Heparin-protamine Complexes and C-reactive Protein Induce Activation of the Classical Complement Pathway: Studies in Patients Undergoing Cardiac Surgery and In Vitro

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Key words
C-reactive protein, heparin, protamine, complement, extracorporeal circulation

Summary
The administration of protamine to patients undergoing cardiopulmonary bypass (CPB) to neutralize heparin and to reduce the risk of bleeding, induces activation of the classical complement pathway mainly by heparin-protamine complexes. We investigated whether C-reactive protein (CRP) contributes to protamine-induced complement activation.

In 24 patients during myocardial revascularization, we measured complement, CRP, and complement-CRP complexes, reflecting CRP-mediated complement activation in vivo. We also incubated plasma from healthy volunteers with heparin and protamine in vitro to study CRP-mediated complement activation. During CPB, CRP levels remained unchanged while C3 activation products increased. C4 activation occurred after protamine administration. CRP-complement complexes increased at the end of CPB and upon protamine administration. Incubation of plasma with heparin and protamine in vitro generated complement-CRP complexes, which was blocked by phosphorylcholine and stimulated by exogenous CRP. C4d-CRP complex formation after protamine administration correlated clinically with the incidence of postoperative arrhythmia.

Protamine administration during cardiac surgery induces complement activation which in part is CRP-dependent, and correlates with postoperative arrhythmia.

Introduction
Complement activation during CPB has been studied extensively and is reported to occur mainly via the alternative pathway, until after protamine administration when significant classical pathway activation occurs in addition (1-3). The contribution of complement activation to the clinical adverse events in patients undergoing CPB is still not well understood although methods to reduce this activation, for example by coating of the extracorporeal circuits with heparin, have been advocated (4, 5). Recently, we described significant complement activation after the CPB-procedure mainly occurring via the classical pathway, i.e. during the acute phase response induced by the operation (6). This activation was shown to be mediated, at least in part, by CRP, as it coincided with increasing levels of CRP-complement complexes. CRP-dependent complement activation indeed has been observed in vitro. For example, already in 1974, Siegel and colleagues (7), reported that CRP enhanced complement activation induced by heparin-protamine complexes. As patients undergoing CPB receive heparin, which at the end of CPB is neutralized with protamine, the classical pathway activation induced by protamine administration, may be at least in part dependent on CRP. Therefore, we conducted a study to investigate the role of CRP in protamine induced activation in more detail. For this we measured complement activation and CRP-complement complex formation in patients undergoing cardiac surgery, in particular before and after protamine administration. In addition, we performed in vitro experiments with plasma obtained from these patients as well as from healthy donors. Our results indicate that part of the complement activation induced by heparin-protamine complexes in patients undergoing CPB is CRP-dependent.

Patients, Materials and Methods
Study Design
We prospectively studied 24 patients (20 men, 4 women) aged 64.6 years of age (median: interquartile range, 58-69.4 years) undergoing CPB for myocardial revascularization. All patients had good left ventricular function and were free of underlying diseases other than coronary artery disease. Patients using corticosteroids or other anti-inflammatory drugs, except for low doses of aspirin, i.e. 80-100 mg, before or during surgery, were excluded from the study. Patients gave written informed consent and were evaluated according to a protocol approved by the Medical Ethics Committee of the Academic Medical Centre of Amsterdam.

Anesthesia was induced intravenously with etomidate (0.2 mg/kg), fentanyl (50 μg/kg), pancuronium bromide (0.1 mg/kg), and midazolam (0.1 mg/kg), and maintained by supplemental doses. A radial artery catheter and a flow-directed pulmonary artery catheter (Swan-Ganz, Baxter/American Edwards Laboratories, Santa Ana, USA) were inserted for hemodynamic measurements and collection of blood samples.

The extracorporeal circuit consisted of a membrane oxygenator with integrated heat exchanger and cardiomyotomy reservoir (Cobe Cardiovascular Inc., Arvada, CO), a non-pulsatile roller pump (Sarns 9000, 3M Health Care Group, Ann Arbor, MI), arterial filter (AF-1025D, Baxter Healthcare Corp, Irvine, CA) and polyvinyl tubing system (Cobe). The extracorporeal circuit was primed with 1 L of Haemaccel® (Hoechst Marion Roussel B.V., Hoevelaken, The Netherlands), 0.5 L of Hartmann (NPBI, Emmer-Compascuum, The Netherlands), and 50 mL of 8.4% sodium bicarbonate (NPBI), 200 mL of aprotinin (2 × 106 KIU Trasylo®; Bayer, Leverkusen, Germany), and 100 mL of 20%, w/v, mannitol. Magnesium sulphate (0.1 gram/ kg) and 5,000 IU bovine hepar-
in (Leo Pharmaceutical Products, Weesp, The Netherlands) were added to the priming solution. Total priming volume was 1,850 mL.

Following systemic heparinization (300 IU/kg), CPB was initiated with cannulas placed in the ascending aorta and right atrium (two-stage single venous cannula). Activated clotting time (ACT) was kept above 500 sec by giving additional heparin, i.e. 10,000 IU per hour. A non-pulsatile roller pump was used for all operations. Patients were cooled to 27-30° C. St. Thomas’ Hospital cold cardiopulmonary resuscitation (Academic Medical Centre, Amsterdam, The Netherlands) was infused into the aortic root, to protect the myocardium during aortic cross-clamping. The hematomix during CPB was maintained at 18-25%. After termination of CPB, heparin was antagonized with protamine hydrochloride (Roche, Basel, Switzerland) at a ratio of approximately 1:1 (3 mg/kg). Autologous blood and residual volume from the extracorporeal circuit were infused into the patient when volume supplementation was necessary.

Following surgery, patients were admitted to the intensive care unit (ICU), and treated according to a standardized clinical protocol. Packed erythrocytes were infused when the hematocrit was less than 26%. Upon stabilization of their cardiorespiratory condition, patients were transported to the ward for further recovery.

Collection of Blood Samples

Blood specimens for hemoglobin, hematocrit, white blood cell numbers, and platelet counts were collected into 4.5 mL glass Vacutainer® tubes containing EDTA (Becton Dickinson, Franklin Lakes, USA). Blood samples for analysis of complement activation products, CRP, and complement-CRP complexes were collected into 4.5 mL siliconized glass Vacutainer® tubes containing 3.8% trisodium citrate solution (0.105 M) (Becton Dickinson). All samples were taken via a radial artery catheter. Plasma was prepared by centrifugation of the blood samples for 20 min at 1500 x g immediately after collection, and stored in aliquots at -70° C. Blood samples were obtained at the following time points: before and after the induction of anesthesia; 30 min after the start of CPB; immediately after CPB, but before protamine administration; and after protamine administration, when the sternum was closed.

In vitro Experiments

Blood was collected from healthy volunteers into a 9 mL siliconized glass Vacutainer® tube containing 3.8% trisodium citrate solution (0.105 M) (Becton Dickinson). Plasma was prepared as described above. Heparin (the same as used in the patients, see above) was added to a final concentration of 0, 2, 4, or 8 IU/mL to glass tubes containing 900 μL aliquots of plasma, and incubated for 30 min at room temperature on a shaker (200 rpm). The mixture was then recalcified by incubation with 10 mM CaCl₂ (final concentration) for 20 min at 37° C, whereafter a clot had formed in the samples without heparin. The mixtures were then further incubated for 30 min on melting ice, and centrifuged for 15 min at 1500 x g at 4° C. The supernatants were then distributed into plastic tubes to yield 5 aliquots. Thereafter, protamine hydrochloride (Roche) was added at final concentrations ranging from 0 to 0.8 mg/mL, and the mixtures were incubated for 20 min at room temperature. Binding of CRP to ligands can be inhibited by phospholipid-choline (8, 9). Therefore, to study the contribution of CRP to the observed complement activation, in other experiments 10 mM β-amino phospholipid-choline (final concentration; Sigma Chemical Co., St. Louis, MO) was added, just before incubation with protamine at a concentration of 0.2 mg/mL. In addition, in other experiments samples were supplemented with additional CRP [purified as described (10)], to yield a concentration of 26.5 mg/L, before adding the protamine. The in vitro experiments described above were done with plasma of healthy volunteers as well as with plasma samples taken at baseline from 4 patients randomly chosen from the 24 patients studied. These samples were supplemented with heparin (final concentrations of 0 to 4 IU/mL), as well as with protamine (0, 0.05, or 0.1 mg/mL), and incubated as described above. Finally, the various mixtures were analyzed for CRP, complement activation products, and complement-CRP complexes as described in the next paragraph. Generation of the various activation products was calculated by subtracting baseline values from observed levels. All experiments were repeated at least once and appeared to be reproducible. Representative examples are given in the results section.

Biochemical Parameters

Heparin levels were determined with a chromogenic anti-Xa assay (Chromogenix AB, Mölndal, Sweden) in an automated Epos Analyzer 5060 (Eppendorf, Nettler-Hinz-GmbH, Hamburg, Germany), following the manufacturer’s instructions (11, 12). Briefly, 100 μL of plasma were diluted with 100 μL of lyophilized human antithrombin III (1 IU/mL), and 800 μL of Tris buffer 0.05 mol/L, pH 8.4, and incubated at 37° C for 3 min. Then, 200 μL of the diluted sample was mixed with 100 μL bovine factor Xa and incubated at 37° C for 30 sec, whereafter 200 μL of chromogenic substrate S-2222 (Br-Ile-Glu(–OR)-Gly-Arg-pNA-HCl), 1 mmol/L, were added. The mixtures were then incubated for exactly 3 min at 37° C, whereafter 300 μL of acidic acid 2% v/v, were added to stop the reaction. The color was read photometrically at 405 nm. Detection limit of the assay was 0.05 IU/mL.

C3b/c (i.e. C3b, C3bi or C3c) and C4b/c (i.e. C4b, C4bi or C4c) levels were determined with ELISAs as described (13-15). Briefly, C3b/c was measured using monoclonal antibody (mAb) anti-C3-9 as capture antibody, which binds to C3b, iC3b, C3c as well as iC3 (C3b-like C3) (16). Biotinylated polyclonal rabbit antibodies to human C3c were used as detecting antibody. C4b/c was measured using mAb anti-C4-1, which binds to a neoepitope exposed on C4b, iC4b, C4c as well as iC4, as capture antibody. Bound C4b/c was detected with biotinylated anti-C4c antibodies. Normal values of C3b/c and C4b/c are below 50 nmol/L.

Total C3 and C4 concentrations were determined by nephelometry (antisera from Behring Diagnostics Benelux N.V., Rijswijk, The Netherlands; Behring Nephelometer Analyzer, Behringwerke AG, Marburg, Germany) according to the manufacturer’s protocol.

C-reactive protein (CRP) levels were measured with a sandwich-type ELISA in which polyclonal rabbit anti-CRP antibodies were used as capturing antibodies and a biotinylated mAb against CRP (CLB anti-CRP-2) as the detecting antibody. Results were related to a standard consisting of commercially available CRP (Behringwerke AG, Marburg, Germany), and expressed as mg/L. The detection limit of the assay was 10 ng/mL. CRP levels in healthy persons are below 3 mg/L.

Complement-CRP complexes were measured with sensitive ELISAs as described previously (10). Briefly, activated complement proteins fixed to CRP were separated from unbound complement proteins by absorption onto phospholipid-choline-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden). For the quantification of C3d-CRP and C4d-CRP complexes, purified mAb anti-C3-19 or mAb anti-C4-4 were used as a catching antibody, respectively. It is to be noted that the ELISAs for C3d-CRP complexes detects C3d-CRP, C3b-CRP and C3b-CRP, and that for C4d-CRP complexes C4d-CRP, C4b-CRP, and C4b-CRP, respectively. Complement-CRP complex levels in healthy volunteers are below 4 pmol/L.

Statistical Analysis

Data were stored and analyzed using standard computer software (SPSS 6.1.3, SPSS Inc., Chicago, IL). To analyze changes in time, one factor analysis of variance (ANOVA) for repeated measurements was applied, supplemented with the Bonferroni post-hoc test. Correlations were determined with Spearman’s rank correlation test. Regression analysis was used to assess correlation between parameters. A two-sided probability value of p <0.05 was considered to be statistically significant. Values are presented as medians with interquartile range (IQR), unless stated otherwise.

Results

Patients

The clinical characteristics of the 24 patients included are shown in Table 1. Heparin concentration at the end of CPB before protamine ad-
administration was 4.8 IU/mL (IQR: 4.6 to 6.2 IU/mL). After protamine administration, ACT returned to baseline levels (preoperative median: 136 s, IQR: 119 to 146 s, versus postoperative median: 125 s, IQR: 112 to 139 s) despite the presence of detectable heparin in 13 of 24 patients (median: 0.19 IU/mL, IQR: 0.1 to 0.34 IU/mL). Protamine-heparin ratio corrected for body surface area was 1.0 (IQR: 0.78 to 1.34). No patient required reoperation for excessive postoperative bleeding. All patients survived the operation and had a normal recovery. Atrial fibrillation with fast ventricular rate occurred in 8 of 24 patients during the first operative days (i.e. 3 patients on day 2, 2 patients on day 3, and 1 patient on days 0, 1 and 4, respectively).

Complement Activation In Vivo

Of the 24 patients enrolled in the study, one was excluded from the biochemical analysis, because of an unexplained elevation of preoperative CRP (31 mg/L) level. This patient will be described separately below. In the other 23 patients C3b/c levels increased significantly after the start of CPB, reaching maximum levels after protamine administration (median: 235 nmol/L, IQR: 177 to 336 nmol/L; Table 2). In contrast, C4b/c levels did not significantly increase until after protamine administration (median: 2.1%, IQR: 1.1 to 2.9%; Table 2).

CRP levels did not change during the procedure (Table 2), but C3d-CRP levels significantly increased at the end of CPB (from <4 pmol/L to median: 13 pmol/L, IQR: 5.3 to 25 pmol/L; Table 2) and remained elevated after protamine administration (median: 16 pmol/L, IQR: 5.5 to 51 pmol/L). C4d-CRP levels were already slightly increased at the end of CPB (median: 6.2 pmol/L, IQR: 2.2 to 12 pmol/L), but became elevated dramatically after protamine administration (median: 27 pmol/L, IQR: 9.8 to 87 pmol/L). There was no difference in complement activation after protamine administration between patients with or without detectable levels of heparin after protamine administration.

The patient who, because of elevated baseline-CRP level was excluded from statistical analysis, showed an illustrative CRP-mediated complement activation. Baseline CRP level was 31 mg/L. After protamine administration CRP decreased to 20 mg/L. C3d-CRP level increased from baseline 20 to 279 pmol/L at the end of CPB and after protamine administration to >3500 pmol/L. C3 activation was comparable with that in the other patients, but C4b/c increased from baseline level of 2.6 to 34 nmol/L after protamine administration. In the postoperative period this patient did not suffer from arrhythmia.

In Vitro Studies

The results of the in vitro experiments with plasma of healthy volunteers are shown in Figs. 2 to 4 (data presented are medians with range (n = 3). Heparin-protamine complexes stimulated C3 activation only slightly at high heparin concentrations, but induced significant C4 activation at 2 U/mL heparin (not shown). C4b/c generation appeared to be the highest at optimal antagonizing concentrations of protamine for heparin activity. Additional CRP had no effect on C4 activation, but induced a slight activation of C3, independently of the administration of protamine (not shown). The rise of activation products specifically reflecting CRP-dependent activation, i.e. CRP-complement complexes,
in these in vitro experiments was more remarkable. CRP-complement complex formation depended on both heparin and protamine dose (Fig. 1). At optimal antagonizing concentrations of protamine the highest levels of C3d-CRP and C4d-CRP complexes were formed. Raising the CRP concentration about 10-fold increased the CRP-complement complex levels also about 10-fold, but did not alter the optimal heparin-protamine ratios to stimulate complex formation (Fig. 1). We did several control experiments to demonstrate the specificity of the observed CRP-dependent activation. In the presence of 10 mM EDTA no activation was observed (not shown). Furthermore, addition of phosphorylcholine completely prevented C4d-CRP and C3d-CRP complex formation (Fig. 2A and B).

We also incubated baseline plasma of 4 randomly chosen patients with different concentrations of heparin and protamine. Results were comparable with those obtained with normal plasma, though in the plasma of two of these four patients more C4b/c and complement-CRP were generated compared to normal plasma: i.e. after incubation with 4 IU/mL of heparin and 0.1 mg/mL of protamine, for C4b/c 0 and 24 nmol/L vs. 43 and 123 nmol/L, for C4d-CRP 201 and 254 pmol/L vs. 4698 and 7714 pmol/L, and for C3d-CRP 1.8 and 18 pmol/L vs. 352 and 642 pmol/L (not shown).

**Table 3** Complement activation at various points of time in the patients with or without arrhythmia

<table>
<thead>
<tr>
<th>Sample point</th>
<th>Arrhythmia (n=8)</th>
<th>No Arrhythmia (n=15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3b/c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>13 (11 - 14)</td>
<td>11 (9.4 - 14)</td>
<td>NS</td>
</tr>
<tr>
<td>after protamine</td>
<td>196 (181 - 258)</td>
<td>243 (156 - 354)</td>
<td>NS</td>
</tr>
<tr>
<td>- fold increase</td>
<td>17 (14 - 22)</td>
<td>19 (12 - 35)</td>
<td>NS</td>
</tr>
<tr>
<td>C4b/c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>3.7 (2.8 - 9.0)</td>
<td>3.3 (1.9 - 15)</td>
<td>NS</td>
</tr>
<tr>
<td>after protamine</td>
<td>10 (7.5 - 13)</td>
<td>11 (7.7 - 18)</td>
<td>NS</td>
</tr>
<tr>
<td>- fold increase</td>
<td>2.2 (0.9 - 4.6)</td>
<td>4.9 (0.8 - 6.8)</td>
<td>NS</td>
</tr>
<tr>
<td>C3d-CRP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>1.4 (0.3 - 2.6)</td>
<td>2.3 (ND - 3.8)</td>
<td>NS</td>
</tr>
<tr>
<td>after protamine</td>
<td>17 (8.5 - 51)</td>
<td>16 (4.9 - 51)</td>
<td>NS</td>
</tr>
<tr>
<td>- fold increase</td>
<td>9.1 (5.6 - 13)</td>
<td>4.9 (3.0 - 16)</td>
<td>NS</td>
</tr>
<tr>
<td>C4d-CRP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>3.0 (1.9 - 6.2)</td>
<td>2.9 (1.0 - 8.8)</td>
<td>NS</td>
</tr>
<tr>
<td>after protamine</td>
<td>37 (16 - 82)</td>
<td>19 (5.8 - 135)</td>
<td>NS</td>
</tr>
<tr>
<td>- fold increase</td>
<td>13 (6 - 17)</td>
<td>3.8 (2.9 - 7.0)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data are expressed as median values (interquartile range). Differences between the two groups were determined by Mann-Whitney U-test. ND = not detectable, NS = Not Significant (p>0.05).

**Correlation between Complement Parameters and Clinical Symptoms**

In the 23 patients, we found a significant correlation between baseline levels of C3d-CRP and C4d-CRP and the respective peak levels after protamine administration (r = 0.60, p = 0.002 and r = 0.77, p <0.001, respectively; Fig. 3A and B). The baseline levels of CRP also correlated with the peak levels of C3d-CRP and C4d-CRP (r = 0.72 and r = 0.65, respectively; all p <0.001) (Fig. 4). The median increase of C4d-CRP after protamine administration was 6-fold compared to baseline levels, but in individual patients this increase ranged from 2.9- to 17-fold. The extent of this increase was associated with the occurrence of arrhythmia postoperatively (r = 0.59, p = 0.007), whereas absolute peak levels of C4d-CRP did not correlate, nor did the increases of C4b/c or C3d-CRP, although the increase of C4b/c tended to be higher in patients with arrhythmia (Table 3). To explain maximum C4d-CRP levels we entered the following independent variables in a forward stepwise regression analysis: baseline of total C3, C4, C3b/c, C4b/c, CRP, and C4d-CRP; patient’s age; leucocyte count at the end of CPB;
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CPB and cross-clamp time; protamine dose, and protamine/heparin ratio corrected for body surface area. The maximum level of C4d-CRP was mainly explained by two variables: C4d-CRP after protamine = \(-19.8 + 3.1 \times \text{Baseline C4d-CRP} + 4.95 \times \text{leucocyte count at the end of CPB}\); the adjusted R² for this model was 0.75 (p <0.001). The confidence interval for total C4d-CRP at baseline was 2.2-4.0; and that for leucocyte count at the end of CPB, 0.15 to 9.8.

**Discussion**

Previous studies have shown that heparin-protamine complexes enhance classical pathway activation in vivo (2, 18, 19). Here we show that at least part of this activation is mediated by CRP. This CRP-dependent activation of complement appeared to be clinically relevant since it was associated with the occurrence of arrhythmia (6). Whether this association reflects a direct role in the pathogenic processes leading to arrhythmia is unclear. Griselli and colleagues (20) recently showed that injection of human CRP into rats after ligation of a coronary artery enhanced infarct size in a complement-dependent fashion, indicating that CRP indeed can contribute to tissue damage by activating complement.

As parameters for CRP-dependent complement activation we measured complement-CRP complexes using assays for complexes of C4b/d or C3b/d to CRP (10). These complexes are specific for complement activation mediated by CRP as they are not generated during activation of complement by other activators (21, 22). Thus, the increased levels of complement-CRP complexes following the administration of protamine is indicative for CRP-dependent activation by heparin-protamine complexes. The in vitro experiments indeed supported the specificity of the generation of complement-CRP complexes, since this generation was completely blocked by phosphorylcholine, and appeared to be Ca²⁺-dependent, which are typical features for CRP-dependent complement activation.
Fig. 1 shows that various concentrations of either heparin or protamine resulted in different levels of complement-CRP complexes and that an optimal ratio of either agent was needed for maximal activation. In normal plasma the optimal ratio was 4 IU/mL heparin and 0.1 mg/mL protamine. Hence, polymerization of heparin by protamine likely is a key event to favor binding of CRP and subsequently activation of complement. Notably, these in vitro experiments in plasma were performed at similar concentrations as occur in vivo. During CPB-mediated complement activation in vitro approximately 1 to 5% of activated C4 and C3 will fix to CRP (10). We estimated that 0.008% of activated C3 and 0.26% of activated C4 became fixed to CRP during CPB. This lower ratio in vivo may be explained by a more rapid clearance of complement-CRP complexes bound to heparin-protamine, as compared to free activated C3 or C4. Alternatively, it could imply that other mechanisms in addition to those involving CRP, account for the protamine-induced activation of complement.

Maximum levels of C3d-CRP were found at the end of CPB and increased further though not significantly after protamine administration, whereas levels of C4d-CRP increased slightly at the end of CPB and peaked after protamine administration. So already during CPB, i.e. after heparinization, but before protamine administration, some CRP-dependent complement activation occurred, possibly by binding of CRP to for example damaged cells (23). Although our study does not reveal the specific ligand for CRP, we suggest that phospholipids, e.g. lyso-phospholipids generated from phosphatidylcholine of flip-flopped cells by secretory PLA-2, provide a suitable ligand for CRP (23, 24). However, other ligands such as polycations, including leucocyte cationic proteins, histones, polymers of L-lysine, protamine, and phosphatidylcholine, may have contributed to CRP-dependent complement activation (23, 25, 26).

During CPB the human body and especially the heart is exposed to ischemia-reperfusion injury, which induces an inflammatory response (27, 28). We showed recently significant complement activation during the first 3 postoperative days following CPB (6). This second phase of complement activation appeared to be CRP-dependent and was associated with the occurrence of arrhythmia. In this study, we also found an association between the increase in C4d-CRP levels and the occurrence of arrhythmia, but in contrast to the previous study, which concerned CRP-dependent activation in the postoperative days, in this study we focussed on the activation following protamine administration. Interestingly, we found a significant correlation between baseline C4d-CRP complexes and the increase of these complexes following administration of protamine. These data may point to interindividual differences in responsiveness regarding CRP-dependent complement activation. Hence, we suggest that the association between CRP-dependent complement activation following protamine administration and arrhythmia is due to the fact that larger increases following protamine identify high-responders, that will have higher postoperative CRP-dependent activation, the latter activation process being linked with the occurrence of arrhythmia. We are currently testing this hypothesis.

One patient was excluded from analysis, because of elevated baseline CRP level (31 mg/L). In this patient a 10-fold increase in C3d-CRP level and a 4-fold increase in C4d-CRP level occurred at the end of CPB, whereas after protamine administration levels were 90-fold (C3d-CRP) and more than 90-fold increased (C4d-CRP). Baseline levels of total C3 and C4, and complement activation products were normal. At the end of CPB C3b/c and C4b/c levels showed 12.5-fold and 2-fold increase, and after protamine administration increases were 23-fold and 13-fold, respectively. Hence, we speculate that patients with higher CRP levels have stronger complement responses than normal individuals. This is consistent with the in vitro observation that elevated CRP levels are accompanied by more extensive activation patterns. In conclusion, we show that activation of the classical pathway induced by protamine administration in the presence of heparin, is in part mediated by CRP. Moreover, the extent of this CRP-mediated complement activation is in part dependent on the concentration of CRP. Finally, we found evidence for interindividual differences in the extent of CRP-mediated complement activation, i.e. high responses being associated with the occurrence of arrhythmia in the postoperative period. We suggest that monitoring of the response induced by protamine, may help to identify patients at risk for developing arrhythmia.

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References


