Peri-operative myocardial tissue injury and the release of inflammatory mediators in coronary artery bypass graft patients


*Department of Cardiothoracic Surgery, University Hospital Maastricht, Maastricht, The Netherlands

Methods: Serum levels of enzymes (CK and CK-MB) and non-enzymatic proteins (FABP and myoglobin) as markers of myocardial tissue injury, bactericidal permeability increasing protein (BPI) as an indicator of neutrophil activation, interleukin-6 (IL-6) as inducer of the acute phase response and lipopolysaccharide binding protein (LBP) as parameter of the acute phase response were measured in 15 low-risk CABG patients with cardiopulmonary bypass (CPB), and 17 low-risk CABG patients without CPB.

Results: Already 0.5 h after reperfusion significantly increased plasma levels of all markers of myocardial tissue injury were noted in patients having surgery with CPB, but not in non-CPB patients. No significant differences were found between both groups for BPI and IL-6 levels in the early reperfusion period. BPI and IL-6 levels were higher in the non-CPB group on the first post-operative day ($P < 0.05$). However, no correlations were found for any marker of peri-operative tissue damage with either early neutrophil activation, or acute phase reactants.

Conclusions: Perioperative myocardial injury resulting from CPB and aortic cross-clamping in low-risk CABG patients does not contribute to the release of inflammatory mediators in these patients.

Keywords: Cardiovascular surgery; Ischemia; Reperfusion; Enzyme (kinetics); Cytokines

1. Introduction

In patients undergoing coronary artery bypass grafting (CABG), the use of cardiopulmonary bypass (CPB) in combination with aortic clamping during coronary artery bypass grafting (CABG) elicits ischemic myocardial injury [1]. Furthermore, these patients experience systemic inflammation leading to an acute phase response with sepsis-like symptoms during post-operative recovery [2,3]. Experimental models of ischemic myocardial injury and observations in patients with myocardial infarction indicate that ischemic myocardial damage is generally associated with inflammation and the release of inflammatory mediators [4–7]. Therefore, in the present study, we hypothesized that ischemic myocardial injury as occurring in CABG patients operated on with CPB and aortic cross-clamping, may be associated with the release of inflammatory mediators in these patients.

Measuring markers of myocardial tissue injury and inflammatory mediators in patients undergoing CABG with or without CPB might answer the question as to what extent the myocardial tissue injury resulting from the CPB and aortic cross-clamping procedures contributes to the systemic inflammatory response in these patients. Therefore, we compared the release into plasma of cardiac marker proteins, and of mediators of inflammation in...
patients undergoing CABG either without CPB, or with normothermic CPB (including aortic cross-clamping). In these patients we measured creatine kinase (CK), creatine kinase-MB (CK-MB), fatty acid-binding protein (FABP) and myoglobin as markers of myocardial tissue loss. In addition, we measured bactericidal permeability increasing protein (BPI) as an indicator of polymorphonuclear neutrophil (PMN) activation, interleukin(IL)-6 as an inducer of the acute phase response and lipopolysaccharide binding protein (LBP) as parameter of the acute phase response. Using these data we were able to examine the relation between global ischemia and reperfusion and the systemic inflammatory response.

2. Methods

2.1. Patients

A total of 32 adult low-risk patients undergoing elective CABG either with or without the use of cardiopulmonary bypass (CPB group (n=15) and non-CPB group (n=17), respectively), were enrolled. In the non-CPB group, no more than two distal anastomoses were made. There were no differences in pre-operative medical treatment with aspirin or other drugs with anti-inflammatory actions between both patient groups. None of the patients received allogeneic packed red cells intra-operatively. The investigation conforms with the principles outlined in the Declaration of Helsinki.

2.2. Intra-operative patient management

Standard anesthetic (lorazepam, fentanyl citrate, sufentanil citrate, alfentanil hydrochloride, midazolam hydrochloride, pancuronium bromide) and monitoring techniques (electro-cardiogram, central venous/pulmonary and arterial pressure monitoring, urinary output, rectal and skin temperature monitoring) were used in all patients. Before connection of the extracorporeal circuit for cardiopulmonary bypass, heparin was administered (300 IU/kg, Heparin Leo, Leo Pharmaceutical Products BV, Weesp, The Netherlands) in order to achieve an activated coagulation time (ACT) > 480 s (Hemochron 400, International Technidyne Corp., New Jersey, USA). Specifications on the extracorporeal circulation circuit, cardiopulmonary bypass procedures and surgical procedures have been described previously [8]. In CPB patients, target flow rates of 2.4 to 2.6 l/min per square meter were maintained. In case of low mean arterial pressure, phenylepinephrine or aramine was administered. During the whole CPB procedure (including the period of aortic cross-clamping), the arterial blood temperature was kept at approximately 36°C, and attention was given to keep the right heart empty. In non-CPB patients, after median sternotomy, coronary grafting was performed on a beating, normothermic heart. To dampen the movement of the beating heart and consequently isolate the region for anastomosis, a custommade, U-shaped stabilizer was used. Through its shaft, the stabilizer was attached to a slightly adjusted sternal retractor. Using vessel loops, a segmentary occlusion of the coronary artery to a length of ±2.5 cm was used to control bleeding from the coronary artery during the anastomy procedure. Postoperative patient treatment in the coronary care unit was standardized and similar for all groups. None of the patients received thrombolytic agents.

2.3. Blood sampling

Blood samples in the CPB group were taken upon induction of anesthesia, and at 0.5, 1.5, 4, 8, 12, 18 h after the start of reperfusion. In the non-CPB group, samples were taken upon induction of anesthesia and 0.5, 1.5, 4, 8, 12 and 18 h after unclamping the internal mammary artery or the venous graft (start of reperfusion). Samples at 1.5 and 12 h after the start of reperfusion were collected only for enzyme measurements. All samples were taken from the central venous line. For enzyme and cardiac marker protein measurements, samples were collected in Corvac integrated serum separator tubes (10 ml, Corvac, Shrewsbury Medical, St. Louis, MO, USA). For inflammatory mediator measurements, samples were collected in evacuated blood collection tubes (10 ml, Monoject, Shrewsbury Medical, Ballymoney, N. Ireland) containing ethylenediamine-tetraacetic acid. Immediately after sampling, blood was cooled, routinely centrifugated, and serum samples were stored at −80°C until assay.

2.4. Analytical techniques

The activities of CK and CK-MB were measured spectrophotometrically at 25°C in a centrifugal analyzer (Cobas Bio Systems, Hoffmann La Roche, Basel, Switzerland) with commercially available test kits. The CK-MB assay is based on immunoinhibition of the predominant M unit in creatine kinase (Boehringer Mannheim, Germany). Serum FABP concentration was measured with a sensitive non-competitive enzyme-linked immunosorbent assay of the antigen capture type (sandwich ELISA) [9]. Serum myoglobin was measured with a turbidimetric immunoassay (Unimate 3 MYO, Roche Diagnostics Systems, Basel, Switzerland) on a Cobas Mira Plus analyzer (Roche).

Plasma levels of BPI, IL-6 and LBP were measured using sandwich enzyme-linked immunosorbent assays (ELISAs), which have been described elsewhere. In short, 96-well plates (Immu-no-Maxisorp; Nunc, Roskilde, Denmark) were coated overnight at 4°C with the appropriate antibodies and free sites were blocked with 1% bovine serum albumin in PBS. Samples and standard dilution series were added for 2 h. For measurement of BPI [10],
human BPI-specific monoclonal antibody 4E3 was used for coating. Human recombinant BPI (kindly provided by M. Marra, Incyte, Palo Alto, CA) was used for standard titration curves. Washing and dilution buffers contained 80 mM magnesium chloride to prevent disturbance by lipopolysaccharide. Biotinylated polyclonal rabbit anti-human BPI IgG was used as detection antibody. The detection limit for the BPI-assay was 200 pg/ml. For the IL-6 ELISA [11], plates were coated with the murine monoclonal antibody 5E1. Human rIL-6 (a kind gift from Prof. W. Sebald, Psychiologisch–Chemisches Institut der Universität Würzburg, Germany) was used for standard titration curves. Biotinylated polyclonal rabbit anti-human IL-6 antiserum was used for detection. IL-6 could be detected with a lower limit of 10 pg/ml. Polyclonal anti-human LBP IgG was used as coating for the LBP ELISA [12]. Human recombinant LBP (provided by M. Marra, Incyte) was used for standard titration curves. Washing and dilution buffers contained 40 mM magnesium chloride to prevent disturbance by lipopolysaccharides. Detection occurred with a biotinylated polyclonal rabbit anti-human LBP IgG. The detection limit was 500 pg/ml. Biotinylated antibodies were detected with peroxidase-conjugated streptavidin (Zymed, San Francisco, CA, USA). Finally, 3,3’5,5’-tetramethylbenzidine (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA) was used as a substrate. Photometry (450 nm) was performed using a micro-ELISA autoreader. All plasma samples were analyzed in the same run.

2.5. Data analysis

All data are presented as mean±standard error of the mean (S.E.M.). A Mann–Whitney U-test was used for comparisons between two variables at the same time point. A Wilcoxon Matched-Pairs Signed-Ranks Test was used for comparisons of values from one variable between two time points. A repeated-measures analysis of variance was used to compare changes in time between both patient groups. A χ²-test was used to test non-numeric variables. The level of significance was set at P-values lower than 0.05.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Perioperative characteristics of the two patient groups*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPB (n=15)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>58±2</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.91±0.1</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6/9</td>
</tr>
<tr>
<td>Cardiopulmonary bypass duration (min)</td>
<td>32±2</td>
</tr>
<tr>
<td>Aortic cross-clamping duration (min)</td>
<td>18±2</td>
</tr>
<tr>
<td>Duration of surgery (min)</td>
<td>131±6</td>
</tr>
<tr>
<td>Number of grafts</td>
<td>1.7±0.1</td>
</tr>
</tbody>
</table>

* Data are presented as mean±S.E.M. BSA, body surface area; ns, non-significant.
addition, at the first post-operative day, BPI levels were significantly higher in the non-CPB group ($P<0.05$).

The acute phase response in both patient groups was characterized by a significant increase of LBP preceded by an increase of IL-6 (Fig. 2, panels B and C). Baseline levels of IL-6 were the same in both groups (Fig. 2, panel B). In the CPB group, IL-6 levels increased and peaked at 8 h after reperfusion (1.8 times higher than baseline levels) ($P<0.05$). In the non-CPB group, IL-6 levels decreased during surgery and then also peaked (2.1 times higher than baseline levels) at 8 h after reperfusion. At the first post-operative day, IL-6 levels were significantly higher in non-CPB patients (Mann–Whitney U-test).

Mean pre-operative plasma levels of LBP were 32.1±4 μg/ml in the CPB group, and 25.1±2 μg/ml in the non-CPB group (Fig. 2, panel C). LBP levels in both groups decreased early after the start of reperfusion, and subsequently were increased at 8 h after reperfusion and on the first post-operative day. LBP levels were significantly higher in CPB patients at 4 h after reperfusion ($P<0.05$).

### 3.4. Possible correlations of markers of myocardial tissue injury and inflammation

In order to examine the correlation of peri-operative myocardial tissue injury with the inflammatory response, we first studied correlations of enzyme and cardiac marker protein levels at 0.5 h after the start of reperfusion, with early neutrophil activation (reflected by BPI levels at 0.5 h after reperfusion). Table 2 shows that no correlations were observed for any marker of peri-operative myocardial tissue damage with early neutrophil activation.

Next, we investigated the correlation of peri-operative myocardial tissue damage on the acute phase reactants. For this, we correlated cardiac marker protein levels at 0.5 h after the start of reperfusion with highest mean plasma levels of IL-6 and LBP. As was found for early neutrophil activation, no correlations were observed for markers of peri-operative myocardial tissue damage with acute phase reactants (Table 2).

To further clarify the correlations of markers of peri-operative tissue damage and inflammatory mediators, Fig. 3 shows scatterplots of CK-MB (as an example) and BPI, IL-6, and LBP. These plots show that whereas CK-MB plasma levels early after the start of reperfusion (x-axis) are higher in CPB patients (filled circles) than in non-CPB patients (open circles), BPI, IL-6, and LBP levels are within the same range (y-axis) in both groups (see also Fig. 1, panel B, and Fig. 2), thus ruling out a correlation between myocardial tissue injury and inflammation.

---

**Fig. 1.** Mean serum concentrations of enzymes CK and CK-MB (panels A and B) and cardiac marker proteins FABP and myoglobin (panels C and D) pre-operatively, and at 0.5, 1.5, 4, 8, 12, and 18 h after the start of reperfusion in low-risk patients who underwent CABG with CPB (black circles) or without CPB (white circles). CK, creatine kinase. CK-MB, creatine kinase isoform MB. FABP, fatty acid-binding protein. PRE, pre-operative at induction. * Indicates $P<0.05$, white circles vs. black circles.
4. Discussion

Myocardial ischemia and reperfusion is a common occurrence in CABG patients. Reintroduction of oxygen to previously ischemic myocardium can result in irreversible tissue injury. Since ischemic myocardial damage is associated with inflammation [4–7], it is tempting to assume that such injury is associated, at least to some extent, with the systemic inflammatory response that is generally found in patients undergoing cardiac surgery. However, this hypothesis is based on the assumption that the CPB procedure, in combination with aortic cross-clamping, is the dominant inducer of systemic inflammation. Yet, in a recent study, we showed that it is not the CPB procedure (including aortic cross-clamping) but the surgical procedure per se (intra-operative trauma and/or anesthetics) that predominantly induces the systemic inflammatory response to CABG patients [13]. Therefore, in the present study we examined whether myocardial ischemia-reperfusion injury, as occurring in CABG patients operated on with CPB, is associated with the release of inflammatory mediators.

4.1. Markers of peri-operative myocardial tissue injury

In the present study, non-CPB patients showed no immediate post-operative rise of cardiac marker protein

---

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>BPI</th>
<th>IL-6</th>
<th>LBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>0.22</td>
<td>0.14</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>(0.26)</td>
<td>(0.47)</td>
<td>(0.91)</td>
</tr>
<tr>
<td>CK-MB</td>
<td>0.36</td>
<td>−0.16</td>
<td>−0.16</td>
</tr>
<tr>
<td></td>
<td>(0.06)</td>
<td>(0.41)</td>
<td>(0.39)</td>
</tr>
<tr>
<td>FABP</td>
<td>−0.14</td>
<td>0.35</td>
<td>−0.24</td>
</tr>
<tr>
<td></td>
<td>(0.48)</td>
<td>(0.86)</td>
<td>(0.21)</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>0.04</td>
<td>0.21</td>
<td>−0.11</td>
</tr>
<tr>
<td></td>
<td>(0.85)</td>
<td>(0.28)</td>
<td>(0.57)</td>
</tr>
</tbody>
</table>

*Values are correlation coefficients (probability values given in parentheses). BPI, bactericidal permeability-increasing protein; CK, creatine kinase; CK-MB, creatine kinase isoform MB; FABP, fatty acid-binding protein; IL-6, interleukin-6; LBP, lipopolysaccharide binding protein.
levels in plasma, but only a relatively slow increase. In contrast, in CPB patients all markers showed an immediate increase during the early hours after the start of reperfusion. At 0.5 h after the start of reperfusion, plasma levels of all markers of peri-operative myocardial tissue injury were significantly higher in CPB patients than in non-CPB patients. Therefore, we conclude that measuring activities of CK and CK-MB and concentrations of FABP and myoglobin in plasma of low-risk CABG patients enables estimation of myocardial injury early after surgery. These data confirm our previous findings [1] also for this group of patients, and thus, in the present study we used plasma levels of these markers at 0.5 h after reperfusion to estimate peri-operative myocardial injury.

4.2. Mediators of inflammation

Activated neutrophils are thought to play a major role in ischemia-reperfusion injury [14]. Therefore, as in previous studies [13,15], in the present study we used BPI as a marker of PMN activation. Patients operated on with CPB in the present study showed a significant increase in BPI levels during the early post-operative phase (Fig. 2, panel A), whereas BPI levels in non-CPB patients did not increase in response to surgery. Remarkably, BPI levels in non-CPB patients were significantly increased from pre-operative levels at the first post-operative day, and at this timepoint were even significantly higher than in CPB patients. In contrast to previous findings, BPI levels early after reperfusion did not significantly differ between both patient groups. The CPB patients in the present study had shorter CPB and aortic cross-clamping times (Table 1). Therefore, the lower BPI levels in the present study might be associated with a shorter contact time with the extracorporeal corporeal circuit.

In agreement with previous studies [13,15], increased plasma levels of IL-6 were observed in both patient groups (Fig. 2, panel B). However, unlike previous findings, a significant delay in IL-6 release in non-CPB patients was not found in the present study. Remarkably, as was the case for BPI levels, IL-6 levels were significantly higher at the first post-operative day in non-CPB patients. Both findings suggest an increased inflammatory activation in non-CPB patients at the first post-operative day, and need further investigation since this may be associated with post-operative morbidity. Levels of the acute phase protein LBP were similar in both patient groups, except for LBP levels at 4 h after the start of reperfusion (Fig. 2, panel C).
Therefore, the present data further confirm our previously formulated hypothesis that it is predominantly the surgical procedure and not the CPB procedure that triggers systemic inflammation in low-risk CABG patients.

4.3. Correlations of markers of myocardial tissue injury and inflammation

As outlined above, in the present study, CPB patients showed significantly higher plasma levels of markers of peri-operative myocardial tissue injury early after the start of reperfusion. Yet, the release of inflammatory mediators was similar in both patient groups. In combination with the data shown in Table 2, these data indicate that in the present group of low-risk CABG patients, peri-operative myocardial tissue injury was not significantly associated to the systemic inflammatory response found in these patients.

Experimental studies on myocardial ischemia-reperfusion injury, using isolated animal hearts, suggest that myocardial ischemia and reperfusion is associated with inflammation [16,17]. In the present study, in low-risk CABG patients, we did not find such an association. Possibly, the experimental findings, under ideal and standardized circumstances, may be hard to substantiate in the clinical setting. Furthermore, although cardiac marker protein levels were significantly higher in CPB patients, duration of CPB and cross-clamping might not have been sufficient to elicit a considerable amount of ischemia-reperfusion induced injury [18]. Last, the release of inflammatory mediators as a result of peri-operative myocardial injury may be obscured by subsequent release of these markers resulting from the intra-operative trauma.

Acknowledgements

The authors wish to thank Maurice Pelsers and Marie-Louise Bouwmans for expert technical assistance and Roche Diagnostic Systems (Basel, Switzerland) for providing the myoglobin test kits.

References