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Cytokine Responses in Low-Risk Coronary Artery Bypass Surgery

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Abstract. Inflammatory cytokines have been implicated in myocardial function, severe congestive heart failure and sepsis. The present study tested the hypothesis that cytokine levels are elevated after low-risk coronary artery bypass surgery (CABG), and that they may be associated with postoperative cardiac dysfunction. Twenty male patients undergoing elective CABG in cardiopulmonary bypass (CPB) were studied. Plasma levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-8, and IL-10 were measured before anesthesia induction, 5 minutes after, and 1, 4, and 20 hours after reperfusion to the myocardium. Levels of the MB isoenzyme of creatine kinase (CK-MB) were measured postoperatively. Hemodynamic data were also recorded. Myocardial ischemia was followed by an increase in the plasma levels of IL-6, IL-8, and IL-10. The duration of IL-6 response lasted throughout the postoperative period studied. Plasma cytokine levels at 1 hour after reperfusion correlated with the maximum CK-MB value (IL-6, $r = 0.587$, $p < 0.01$; IL-8, $r = 0.460$, $p < 0.05$; IL-10, $r = 0.570$, $p < 0.05$). Higher plasma IL-6 and IL-8 levels after reperfusion tended to be linked with lower cardiac index. The present results confirm that the levels of inflammatory cytokines IL-6, IL-8, and IL-10 are elevated after CABG. Increased systemic pro-inflammatory cytokine levels were partially associated with postoperative myocardial dysfunction.

Introduction

Coronary artery bypass grafting (CABG) with cardiopulmonary bypass (CPB) frequently induces a systemic inflammatory response involving secretion of cytokines. These have a multitude of biologic consequences, ranging from minor target organ dysfunction to multi-organ failure. The release of cytokines during cardiac surgery can be deleterious to the heart and other organs. Recent studies have suggested that the proinflammatory cytokines have significant cardiovascular activity by regulating nitric oxide homeostasis [1] and mediating interactions between leukocytes and the endothelium [2]. The release of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) [3], interleukin (IL)-1 [4], IL-6, and IL-8 [5,6], is associated with the development of complications after CABG in CPB.

The association between inflammation and myocardial ischemic injury has been recognized for over 50 years, and remains a topic of continued investigation. A better understanding of this process may lead to improved patient outcome by making possible the development of novel therapies aimed at limiting the inflammatory response. The aim of the present study was to investigate the time-course of circulating cytokine levels after CABG, and to establish whether their levels were linked with postoperative myocardial ischemic injury and cardiac function.

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Methods

Patient Selection

The investigation was approved by the local ethics committee and informed written consent was obtained from all patients entering the study. Twenty male patients with multiple-vessel coronary artery disease and stable angina, admitted for the first time for elective coronary artery bypass surgery, were invited to take part, from July to December 1999. Patients with unstable angina, poor left ventricular function (ejection fraction < 30%), valve disease, and those on corticosteriod medication were deemed ineligible. Patients with an aortic cross-clamping time exceeding 120 minutes, and/or any postoperative complication requiring re-exploration were also excluded. There were no major complications in any of the 20 patients. The clinical data are presented in Table 1.

CPB and CABG

In the evening before the operation, the patients received a single dose of lorazepam (2 mg) orally. Anti-hypertensive, anti-anginal, and other cardiac medication was continued up to the day of surgery. Pre-medication consisted of morphine (8–12 mg) and scopolamine (0.2–0.4 mg), given i.m. prior to the induction of anesthesia.

Anesthesia was induced with lorazepam (1–2 mg), thiopentone (2 mg/kg) and fentanyl (7 μg/kg). Pancuronium (0.1 mg/kg) was given to facilitate

Table 1. Clinical data

Number of patients	20
Age (years)	62.20 ± 7.62
Body surface area (m^2)	2.02 ± 0.14
Ejection fraction (%)	61.42 ± 11.69
NYHA (II/III)	7/13
Number of Grafts	4 (2 – 5)*
CPB time (min)	106.30 ± 27.00
Aortic cross-clamping time (min)	79.50 ± 19.05
CK MB 6 hours (U/L)	42.75 ± 20.38
CK-MB 1st day (U/L)	28.15 ± 12.71
CK-MB 2nd day (U/L)	14.85 ± 7.69

Data are shown in mean ± standard deviation
*Median and range
CPB = Cardiopulmonary bypass

intubation, with further increments (0.03 mg/kg) as required to maintain muscle relaxation. After endotracheal intubation, the lungs were ventilated mechanically with oxygen in air (FiO_2 = 0.40) using positive pressure ventilation. During surgery, additional bolus doses of fentanyl (total dose 20 μg/kg) were given to maintain adequate analgesia. Isoflurane was administered to deepen the anesthesia as required during sternotomy. During the CPB phase, no isoflurane was administered.

A standard CABG operation was undertaken with one internal thoracic artery (ITA) and with one to four peripheral vein grafts taken in each case from the lower extremities. The patients were perfused at a temperature of 32°C with nonpulsative flow from a membrane oxygenator (Dideco, Mirandola, Italy). The circuit was primed with 2,000 mL of Ringer acetate. Cold-blood antegrade-retrograde cardioplegia (6–8°C) was delivered through a BCD-Plus device® (Dideco, Mirandola, Italy), which mixed blood with asanguineous solution in a ratio of 4:1. The potassium concentration of the induction cardioplegia was 21 mmol/L. After each distal anastomosis, additional cardioplegic solution was delivered for one minute through the vein graft and a coronary sinus catheter. Proximal anastomoses were completed before aortic declamping. Warm-blood retrograde cardioplegia was given immediately before the end of cross-clamping. After weaning from the CPB, pharmacologic therapy with inotropes and/or vasodilators was used to maintain a cardiac index greater than 2.0 L/min/m^2. Therapeutic decisions on conventional criteria were made independently by the anesthesiologist in charge, who was blind to the study. Corticosteroids or aprotinin were not administered perioperatively.

Sample Collection and Analysis

Blood samples for cytokine measurements were collected from the radial artery before induction of anesthesia (baseline), 5 minutes after, and 1, 4, 8, and 20 hours after myocardium reperfusion. All blood samples were anticoagulated with ethylenediaminetetraacetic acid, immediately cooled to 4°C, and centrifuged within 30 minutes (4000g for 10 minutes); plasma was transferred to polypropylene test tubes and stored at −70°C until assay. TNF-α, IL-6, IL-8, and IL-10 levels in the plasma were determined by means of a commercially available enzyme-linked immunosorbent assay (CLB, Netherlands). The detection limits were 3.0, 0.4, 3.0, and 3.0 pg/ml for TNF-α, IL-6, IL-8, and IL-10, respectively. Creatine kinase cardiac isoenzyme (CK-MB) release was analyzed three times in each patient: 6 hours after reperfusion, and on the first and second postoperative days. All measurements were presented without adjustment for hemodilution.

Hemodynamic Measurements and Data Collection

Hemodynamic monitoring comprised measurements of heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), and cardiac output. Derived cardiovascular variables such as cardiac index (CI), systemic vascular resistance index (SVRI), and pulmonary vascular resistance index (PVRI) were calculated using standard formulas. All measurements were based on the thermodilution technique. Hemodynamic measurements and calculations were collected at three time-points: 1. baseline value, before anesthesia induction, 2. 15 minutes, after, and 3. 6 hours after the end of CPB.

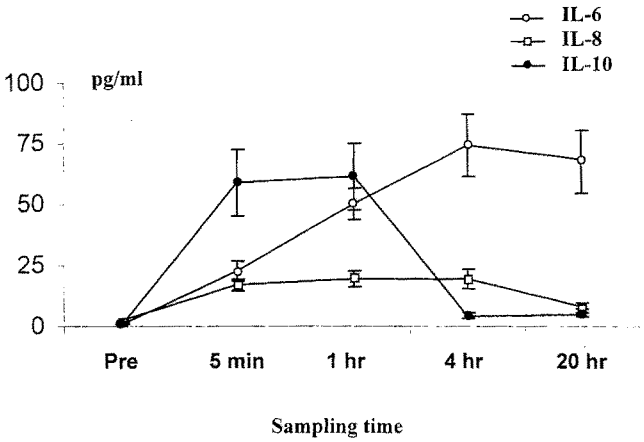

Fig. 1. Perioperative plasma levels of IL-6, IL-8, and IL-10. Before anesthesia induction (Pre), 5 minutes after, and 1, 4, and 20 hours after reperfusion of the myocardium. Data are shown in mean ± standard error of the means.

Statistical Analysis

Results are presented as the mean ± standard deviation (SD) of the mean. For data analysis the analysis of variance for repeated measures was employed. The Mann-Whitney U test was used to compare data between groups. Correlation between different variables was assessed by Pearson's coefficience. Statistical significance was attributed to p values lower than 0.05.

Results

Cytokines

Trace TNF-α (lower than the lowest standard, 3.0 pg/mL) were detected in most of the patients after reperfusion (data not shown). Plasma levels of IL-6 were higher at all time-points as compared to preoperative levels, with the highest increase at 4 hours after reperfusion (Figure 1). IL-8 levels were higher than baseline at 5 minutes, and 1 hour, and 4 hours after reperfusion. However, IL-8 levels at 5 minutes after, and at 1 and 4 hours after reperfusion remained at the same increased level when compared to the baseline values (Figure 1). IL-10 levels rose at 5 minutes and at 1 hour after reperfusion, with the highest level at 1 hour after reperfusion (Figure 1). No correlation was found between cytokine levels and aortic cross-clamping time or CPB time.

CK-MB

CK-MB was higher after the operation, with peak levels at 6 hours (Table 1). It was found that plasma cytokine levels at 1 hour after reperfusion correlated with the maximum postoperative CK-MB value (IL-6, $r = 0.587$, $p < 0.01$; IL-8, $r = 0.460$, $p < 0.05$; and IL-10, $r = 0.570$, $p < 0.05$).

Table 2. Perioperative cytokine release in patients with different change in cardiac index after cardiopulmonary bypass

Time-Points	Group 1	Group 2
Before induction		
IL-6 (pg/ml)	0.73 ± 0.26	0.80 ± 0.33
IL-8 (pg/ml)	1.81 ± 0.97	1.86 ± 1.02
IL10 (pg/ml)	0.62 ± 0.59	0.75 ± 0.66
5 minutes after reperfusion		
IL-6 (pg/ml)	18.79 ± 15.82	23.79 ± 19.81
IL 8 (pg/ml)	14.62 ± 10.11	18.56 ± 9.70
IL10 (pg/ml)	52.09 ± 70.91	62.74 ± 54.56
1 hour after reperfusion		
IL-6 (pg/ml)	39.81 ± 17.62	56.59 ± 32.79
IL-8 (pg/ml)	16.02 ± 10.33	20.79 ± 13.33
IL10 (pg/ml)	74.18 ± 88.06	53.75 ± 32.40
4 hours after reperfusion		
IL-6 (pg/ml)	57.09 ± 25.42	88.63 ± 43.19
IL-8 (pg/ml)	13.82 ± 9.69	22.76 ± 15.91
IL10 (pg/ml)	2.19 ± 2.63	5.12 ± 5.24
20 hours after reperfusion		
IL-6 (pg/ml)	66.03 ± 55.97	68.75 ± 51.03
IL-8 (pg/ml)	7.17 ± 2.83	9.13 ± 5.78
IL10 (pg/ml)	5.67 ± 5.24	3.77 ± 2.60

Data are shown as mean ± standard deviation
Group 1, patients with better or unchanged cardiac index after cardiopulmonary bypass
Group 2, patients with a decrease in cardiac index after cardiopulmonary bypass

Hemodynamics

After CPB, cardiac indexes were improved or unchanged in 8 patients (Group 1) and decreased in another 12 patients (Group 2) compared to the index before anesthesia induction. Mean changes in cardiac indexes were 0.68 ± 0.44 and −0.58 ± 0.54 L/min/m^2 in Groups 1 and 2, respectively. Though none of the differences reached statistical significance, the plasma levels of IL-6 and IL-8 were lower at all study time-points in Group 1 than in Group 2 (Table 2). The absolute CI value did not correlate with circulating levels of measured cytokines perioperatively.

Discussion

It is well established that CABG with CPB induces a systemic inflammatory response [3–6]. The release of cytokines can be stimulated by a number of factors, including ischemia-reperfusion, complement activation, endotoxin release and the effect of other cytokines [7]. The present study confirmed that systemic levels of the inflammatory cytokines IL-6, IL-8, and IL-10 are elevated after CABG, a finding recorded in varying degrees in other studies. Circulating cytokine levels were related to postoperative increase in maximum CK-MB. Plasma levels of IL-6 and IL-8 were higher in patients with decreased cardiac index after CPB. This may indicate that the degree of cytokine production is partially related to the degree of myocardial injury during CABG, although a causal relation remains to be established.

TNF-α has been implicated in many problems arising after cardiac operations with CPB [3,8]. Reports to date on the plasma TNF-α response to CPB have been conflicting. TNF-α may induce endothelial dysfunction with enhanced vascular resistance, fever, hypotension, and leukopenia followed by leukocytosis, hemoconcentration, metabolic acidosis, and circulatory shock [9,10]. In the present study, only traces of TNF-α were found in peripheral plasma, an observation in keeping with some, but not all, previous reports. This may be due to the fact that systemic levels of TNF-α may not properly reflect the local cytokine production in the myocardium, a major source of TNF-α after reperfusion [5].

Plasma levels of IL-6 have been parallel to the severity of tissue damage induced by surgery, and to the inflammatory response to CPB [11]. Echo-cardiographic wall motion abnormalities and postoperative myocardial ischemic episodes have also been associated with increased levels of IL-6 [6]. IL-6 is a sensitive marker of the acute inflammatory response and in cardiac surgery derives mainly from the myocardium [5,12]. The acute-phase response comprises the substantial and diverse systemic and metabolic changes occurring in response to events such as trauma and infection. IL-6 has been thought to play a role in neutrophil-mediated myocardial ischemia-reperfusion injury [13]. The IL-6 response was the longest lasting among the cytokines measured in this study, and significantly increased levels were found even 20 hours after reperfusion.

IL-8 is a potent chemotactic factor which activates neutrophils [14] as well as T lymphocytes, and controls their trafficking [15]. In animal models, the release of IL-8 is induced only after reperfusion of the ischemic myocardium [16,17]. Some investigators have suggested that IL-8 is a critical mediator in inducing myocardial injury due to its correlation with myocardial enzyme release [18]. It has been suggested that the release of IL-8 might be related to more severe myocardial injury [7].

The length of ischemic time has been related to the release of both IL-6 and IL-8 [7,19]. In our study, the ranges of both aortic cross-clamping time and CPB time were narrower than in other studies. For this reason we could not show any significant relationship between cytokine levels and either myocardial ischemic or CPB time. However, the results here indicated that a rise in cytokine level correlated with the maximum CK-MB. This has also been verified in another study [19]. Due to the confounding effects of other factors related to myocardial ischemic damage, a causal relationship between cytokine production and myocardial reperfusion injury is difficult to confirm.

Although none of the differences reached statistical significance, our data showed that higher levels of IL-6 and IL-8 were associated with a decrease in CI. This is in accordance with a previous result obtained when the changes in transesophageal echocardiographic wall motion scores (WMS) were used as the end point [6]. Noteworthy was a trend towards higher cytokine levels in patients with decreased postoperative myocardial function. Baseline cardiac function was independent of circulating proinflammatory mediators, whereas decreased cardiac function was associated with increased levels of IL-6 and IL-8. In human cardiac tissue, IL-6 and TNF-α have a negative inotropic effect [1]. The correlation between systemic cytokine elevation and myocardial ischemic injury and postoperative cardiac function suggested that cytokine release might be partially

associated with cardiovascular instability in patients undergoing CABG. Proinflammatory cytokines may be among the many variables which affect postoperative cardiac function. It is not surprising that only a weak relationship was detected between systemic cytokine levels and myocardial dysfunction. The myocardium has been shown to be a major source of IL-6 and TNF-α after CABG, but systemic cytokine levels are the result of overall cytokine release from different tissues. Plasma cytokine level may thus not properly reflect the local cytokine production; regional myocardial cytokine release may be more important in the mechanism involved in postoperative myocardial dysfunction. The potential effects of myocardial cytokine production on postoperative myocardial dysfunction need to be verified in further studies.

Our data also confirmed a transient elevation in IL-10 after CABG. This occurred simultaneously with the elevation in IL-6 soon after reperfusion. IL-10 is regarded as an anti-inflammatory cytokine. The rise in the plasma proinflammatory cytokines is balanced by this anti-inflammatory cytokine response and this effect was also evident in our study. IL-10 inhibits endotoxin-activated monocyte proinflammatory (TNF-α, IL-1, and IL-6) and anti-inflammatory (IL-10) cytokine production at the transcriptional level [20], while enhancing the release of soluble TNF receptor (which functions to scavenge plasma TNF). It is noteworthy that cytokines are likely to act both individually and within a complex network of interrelated and interacting signals [21]. In contrast to the case with TNF-α and IL-6, the liver is the major source of IL-10 during CABG [22]. Our data showed that plasma levels of IL-10 also correlated with maximum CK-MB. This positive relationship may have been brought out by this balance of pro- and anti-inflammatory cytokine responses. This may indeed be more important in determining the extent of the inflammatory response and the clinical outcome.

Conclusion

In summary, the present study confirmed that peripheral plasma levels of inflammatory cytokines IL-6, IL-8 and IL-10 are elevated after CABG with CPB. Peripheral cytokine levels were weakly related to a postoperative increase in maximum CK-MB. Though there was a trend towards higher peripheral cytokine levels in patients with impaired postoperative myocardial function, systemic cytokines showed no marked correlation with postoperative myocardial function. Regional cytokine levels might be more important in the mechanism involved in myocardial dysfunction. An improved understanding of the cellular biology of this inflammatory response may lead to a clearer conception of adverse postoperative outcomes in cardiac surgery. Further investigation and development of different anti-inflammatory strategies could improve the prospects of patients undergoing cardiac operations.

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