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Effect of Cardiopulmonary Bypass on Cytokine Network and Myocardial Cytokine Production

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Summary

Background: In addition to the well-investigated proinflammatory cytokine expression, there is an ever increasing interest in the field of anti-inflammatory response to cardiopulmonary bypass (CPB). Evidence suggests that myocardium serves as an important source of cytokines during reperfusion and application of CPB. The effect of coronary artery bypass graft (CABG) without CPB on myocardial cytokine production has not as yet been investigated.

Hypothesis: Cardiopulmonary bypass can cause long-term disturbance in pro- and anti-inflammatory cytokine balance, which may impede a patient's recovery following surgery. Therefore, the effect of CPB on the balance of the pro-/anti-inflammatory cytokines network and myocardial cytokine outflow was assessed throughout a longer period after surgery.

Methods: Twenty patients were scheduled for CABG with CPB and 10 had off-pump surgery. Blood samples were taken before, during, and over the first week following surgery. Coronary sinus blood samples were collected during surgery. The ratio of pro- and anti-inflammatory cytokines was calculated and the cytokine concentration of peripheral and coronary sinus blood were compared in both groups.

Results: Pro-/anti-inflammatory cytokine ratio decreased early after CPB followed by a delayed and marked increase. A more balanced ratio was present following off-pump surgery. Coronary sinus levels of certain cytokines exceeded the concentration of systemic blood in the course of CPB but not during off-pump operation.

Conclusion: Patients show pro-inflammatory predominant cytokine balance at a later stage after CPB in contrast to those without CPB. The heart produces a remarkable amount of cytokines only in the course of surgery with CPB.

Key words: coronary artery bypass graft, cardiopulmonary bypass, inflammatory response, myocardial injury, cytokines

Introduction

Recent researches have shown that the activation of acute immune reactions resulting from operative trauma, blood exposure to artificial surface, damage of barrier of intestinal mucosa, abnormal blood gas interfaces, and reperfusion injury after global ischemia of the heart is one of the most formidable aspects of the pathophysiology of cardiopulmonary bypass (CPB).[1–3]

The release of different cytokines regarded as mediators that orchestrate the inflammatory processes, cellular activation, and leukocyte migration, is of central importance. Proinflammatory cytokines in extremely elevated concentrations can modulate the function of organs.[2–5] Dominant anti-inflammatory effects, however, can blunt adequate immune response, impairing defensive mechanisms and healing processes. The balance between pro- and anti-inflammatory cytokines is essential for appraising the genuine effect of different cytokines and the characteristics of the cytokine network. Temporal change of the balance between pro- and anti-inflammatory cytokines has been less investigated.

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Studies have shown that coronary artery bypass graft (CABG) surgery performed without CPB, known as off-pump (OP) surgery, helps to avoid unwanted effects such as overactivation of the inflammatory response.[4]

Evidence suggests that the myocardium is capable of synthesizing biologically active cytokines.[6] The effect of OP surgery on myocardial cytokine production has not as yet been investigated in detail. Therefore, this study investigates the association between CPB or OP surgery and considerable cytokine production by the heart.

Methods

Main Outcome Measures

The main outcome measures in patients who underwent surgery with or without CPB were the pro-/anti-inflammatory cytokine ratio up to the end of first postoperative week, the alteration in interleukin (IL)-12 levels during and after both types of surgery, and the myocardial outflow of cytokines in the course of surgery with or without CPB.

Patients

Thirty patients undergoing elective CABG were selected for the study. The subjects were randomly sorted into two groups: Group 1 consisted of 20 patients who received conventional CABG using CPB and Group 2 of 10 patients who underwent OP surgery. There were no significant differences in the preoperative data of patients (age of patients in Group 1: 62.64 ± 8.76 years and in Group 2: 63.36 ± 5.78 years; three women in Group 1 and two in Group 2; Euro score 2.78 ± 1.56 in Group 1 and 2.6 ± 1.51 in Group 2). Patients with immunologic disease, recent myocardial infarction (< 3 months), previous stroke, or those undergoing acute or repeat surgery, and those who developed infection, coagulopathy, tumor, acute or chronic renal failure, and respiratory impairment were excluded from the study.

The protocol of the study was approved by the Ethics Committee, and oral and written informed consent was obtained from all patients. Investigations were performed in accordance with the Declaration of Helsinki.

Anesthesia and Surgical Technique

Both groups of patients received 10 mg midazolam as premedication. Anesthesia was induced with midazolam 0.1 mg. kg^{-1}, fentanyl 2μg. kg^{-1}, and propofol 2 mg. kg^{-1}. After adequate neuromuscular block with pipecuronium 0.1 mg kg^{-1}, the airway was secured with an endotracheal tube. In patients undergoing CPB, total intravenous anesthesia was maintained with continuous infusion of propofol. After administration of heparin 300 IU kg^{-1}, a hollow fiber oxygenator and a roller pump were used to achieve moderate hypothermic CPB. Myocardial preservation was performed with cold crystalloid cardiolplegia and topical cooling. Heparin was neutralized with protamine sulphate after CPB. In Group 2, anesthesia was maintained with sevoflurane 0.5–1% and nitrous oxide 60% in oxygen. Using Octopus (Medtronic, Inc., Minneapolis, Minn., USA), cardiac stabilizer coronary arteries were occluded for < 20 min.

Blood Sampling

In Group 1, blood samples from peripheral vein were taken right after the induction of anesthesia, then 5 min after the cessation of aorta cross clamping, and on postoperative Days 1, 2, 3, and 7. In Group 2, blood samples were collected after the induction of anesthesia, 5 min after completion of the last graft, and on postoperative Days 1, 2, 3, and 7.

Further blood samples were taken from the coronary sinus (CS) using a catheter in both groups: 5 min after declamping of the aorta in Group 1, and 5 min after the completion of the last graft in Group 2.

All samples were collected in sterile vacuum tubes containing sodium heparin.

Measurement of Cytokines

Blood samples from the peripheral vein were first incubated at 37ºC for 4 h and then stimulated with phorbol 12-myristate13-acetate (PMA, 111 ng ml-1). After this period of stimulation, tubes were centrifuged at 3,000 g for 10 min; then, the supernatant was separated into vials, frozen immediately to −75ºC, and stored at that temperature until the day measurements were taken (within 2 months). The plasma concentrations of stimulated cytokines were determined by using the Becton Dickinson cytometric bead array (CBA Human Inflammatory Kit, BD Biosciences, Pharmingen, Boston, Mass., USA) and by following the instruction manual. This newly developed method allows for reliable simultaneous measurement of six human cytokine levels: tumor necrosis factor (TNF) α and IL –1β, 6, 8, 10, 12p70 (TNF, IL-1, IL-6, IL-8, IL-10, IL-12) from small sample volumes.

Besides the monitoring of the absolute concentration of given cytokines, the pro-/anti-inflammatory cytokine balance was calculated in all samples by dividing the plasma concentration of different proinflammatory cytokines with the concentration of interleukin-10.

During CS blood sampling (in the fifth min of reperfusion), other peripheral blood samples were collected to compare the plasma concentration of cytokines between CS and peripheral vein samples. The PMA stimulation releases cytokines produced by white blood cells. To obtain the cytokines secreted only by the myocardium, PMA stimulation was neglected both in CS and peripheral vein samples. These samples were measured as described above. Cytokine levels of CS blood were not obtained before CPB and ischemia. It is known that without any stimulation or in chronic condition, the level of cytokines CS and systemic, peripheral concentration of cytokines are the same.

The concentration of unstimulated peripheral vein samples was considered to be 100% in each sample. The cytokine levels of CS samples were compared with their corresponding peripheral vein levels, expressing the values in percentage.

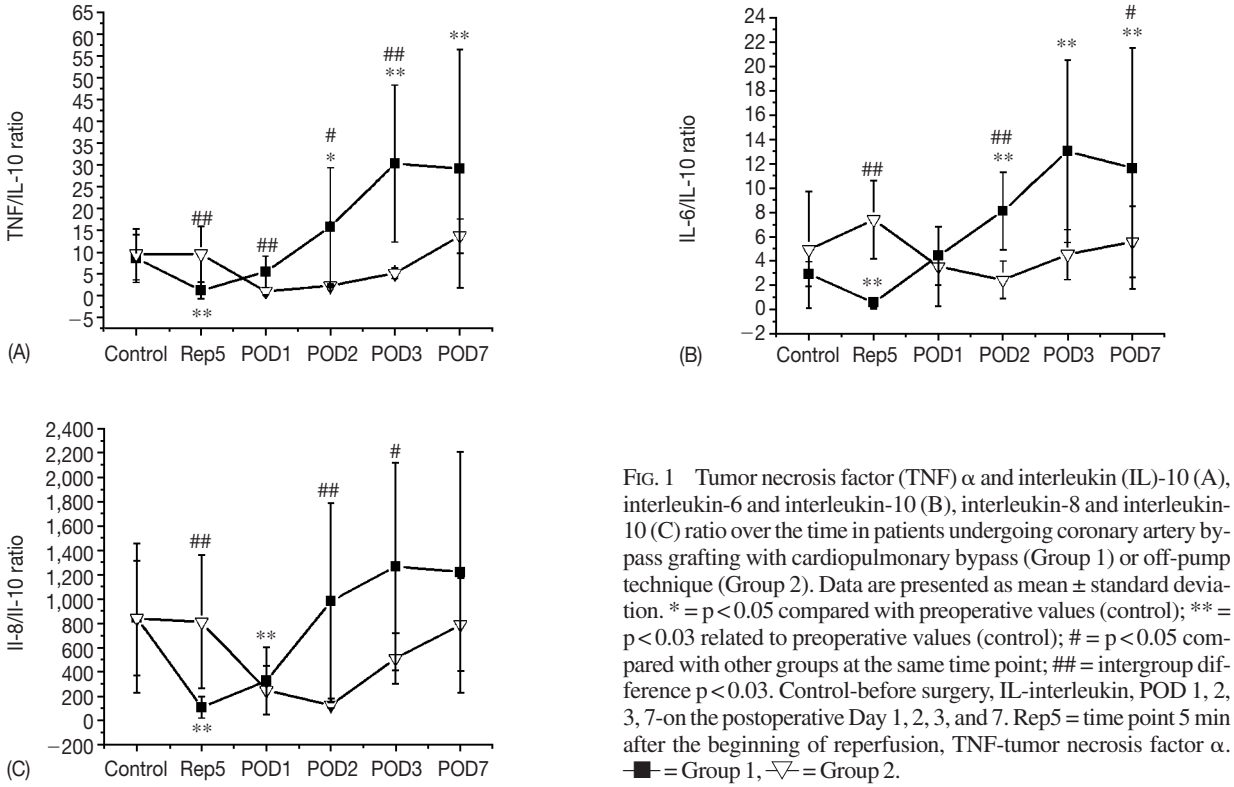

FIG. 1 Tumor necrosis factor (TNF) α and interleukin (IL)-10 (A), interleukin-6 and interleukin-10 (B), interleukin-8 and interleukin-10 (C) ratio over the time in patients undergoing coronary artery bypass grafting with cardiopulmonary bypass (Group 1) or off-pump technique (Group 2). Data are presented as mean ± standard deviation. * = $p < 0.05$ compared with preoperative values (control); ** = $p < 0.03$ related to preoperative values (control); # = $p < 0.05$ compared with other groups at the same time point; ## = intergroup difference $p < 0.03$. Control-before surgery, IL-interleukin, POD 1, 2, 3, 7-on the postoperative Day 1, 2, 3, and 7. Rep5 = time point 5 min after the beginning of reperfusion, TNF-tumor necrosis factor α. —■— = Group 1, —▽— = Group 2.

Statistical Analysis

The data are presented in the Figures as mean ± standard deviation (SD).

The data between the two groups were compared with the unpaired Student's *t*-test. In a given group, comparisons of control data were made using paired Student's *t*-test. Differences were considered significant at p values < 0.05.

Results

There was no hospital mortality pulmonary insufficiency or neurologic complication in either Group 1 or in Group 2. The duration of aortic cross-clamping in Group 1 was 64.13 ± 21.38 min.

Assessment of Cytokine Balance

The balance between inflammatory and anti-inflammatory forces was determined by calculating the proinflammatory cytokine/IL-10 ratio. The ratio was similar in the CPB and OP groups. The TNF/IL-10, IL-6/IL-10, and IL-8/IL-10 ratio is shown in Figure 1A, B, and C. In Group 1, an early drop was observed during surgery, and afterward the ratio increased extremely throughout the observation period. During surgery in the fifth min of reperfusion, the decrease in TNF/IL-10, IL-1/IL-10, IL-6/IL-10, and IL-8/IL-10 ratios was significant when compared with the corresponding preoperative ratios. In Group 2, the ratio of given proinflammatory cytokine and IL-10 tended to decrease, reaching its minimum value on postoperative Days 1 or 2; thereafter, it normalized gradually. Statistical analysis revealed significant differences between Groups 1 and 2, first in the TNF/IL-10 ratio on postoperative Days 1, 2, 3, and 7 (Fig. 1A), then in the IL-6/IL-10 ratio on postoperative Days 2 and 3 (Fig. 1B), in the IL-8/IL-10 ratio in the 5th minute of reperfusion, and on postoperative Days 1 and 2 (Fig. 1C).

Concentration of Cytokines from Samples of Coronary Sinus

In Group 1, all observed cytokines from CS exceeded the concentrations of peripheral vein samples (Fig. 2). The difference between sinus and peripheral vein samples proved to be significant for the IL-1, IL-6, IL-8, and TNF levels.

During OP surgery, the cytokine concentrations of the CS and peripheral vein were roughly equivalent (Fig. 2). It is interesting that the greatest difference was seen in IL-10, although it was not statistically significant.

Discussion

This study shows that CPB caused a prolonged proinflammatory predominant response. The pro-/anti-inflammatory ratio could be balanced by minimal invasive OP technique. The present investigations also demonstrate that the application of CPB and not the OP technique is associated with cytokine production of the myocardium.

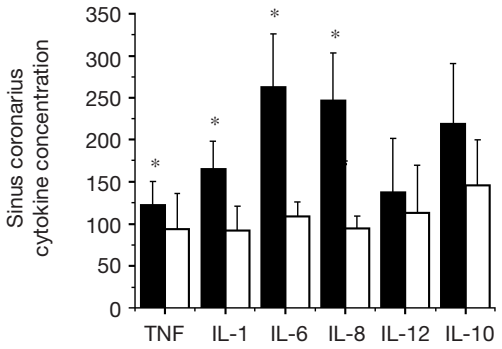

FIG. 2 Cytokine levels of coronary sinus blood during coronary artery bypass graft with cardiopulmonary bypass (Group 1) and with off-pump technique (Group 2). Dark bars show Group 1 and light bars represent Group 2. Concentrations are presented as percent of peripheral venous blood level of given cytokine at the same time. Data are shown as mean ± standard deviation. * = $p < 0.05$ compared with the concentration of peripheral venous blood. ■ = Group 1, □ = Group 2. IL = interleukin, TNF = tumor necrosis factor α.

In this paper, a completely novel method is demonstrated for measuring six cytokines simultaneously from a single sample with cytometry using the cytometric bead array (CBA) technique.[7, 8] This method appears to be an easier, more reliable technique for measuring cytokines compared with conventional enzyme-linked immunosobent assay (ELISA), considering the fact that 300 microparticles are measured for each cytokine, all of them containing approximately the same number of antibodies as are present in one well of an original ELISA kit. Moreover, the CBA technique is highly suitable for assessing the ratio between different cytokines because it can determine six cytokines from the same sample, thereby eliminating certain errors.[8]

Two interesting but rare findings of the present study are the early but significant decrease in TNF and IL-1 concentrations and the late elevation of proinflammatory cytokines. The first can be explained by the extravasation and adherence of active leukocytes to the site of reperfusion and extracorporeal circuit;[1, 3] the latter aspect is the second wave of proinflammatory response, which refers to the potential contribution of mediators, further activating the proinflammatory forces.[9] Evidence is growing to suggest the role such a marker plays in pathomechanism of CPB.[10]

The investigation of IL-12 is of utmost importance as it is known to control type-1 T helper cell (TH1)-mediated immune response. Some articles suggest that IL-12 drops early after the patient is weaned from CPB.[11, 12] According to the findings of this study, the TH1/TH2 response changes in two phases after CPB: first, the TH2-mediated response is stronger subsequently, and second, after postoperative Day 1, the TH1-mediated response tends to be upregulated. Likewise, in the course of OP surgery, it is associated with a more moderate shift in TH1/TH2 response determined by the cytokine pattern.

The absolute concentration of cytokines is considered to be less important than their relative balance, which may better reflect the net effect of cytokine response.[14] The novelty of this study lies in the acknowledgment that the increased and continuously elevating force of proinflammatory response is balanced only during the early postoperative period by IL-10 in patients undergoing CABG with CPB. The results of this study concerning the anti-inflammatory predominant response to CPB up to postoperative Day 1 agree with the outcomes of other studies.[2, 13] Moreover, Hövers-Gürich *et al.* reported similar alterations regarding the later events observing infant patients, specifically the elevation in proinflammatory cytokines without the counterbalance of IL-10.[13] In addition, a lesser alteration in the balance of pro- and anti-inflammatory responses can be observed after OP operation with dominating anti-inflammatory forces. Franke *et al.* found similar results regarding proinflammatory cytokine levels.[14]

These results may have therapeutic consequences. Steroid administration is known to reduce the generation of proinflammatory cytokines with the exaggeration of IL-10 and anti- inflammatory cytokine response, thereby reducing the ratio of pro- and anti-inflammatory cytokines.[15] In most studies examining the efficacy of steroid treatment in patients receiving CPB, steroids were administered before or during surgery.[16] Although the majority of these investigations confirmed the beneficial effect of preoperative steroid treatment, others suggest an adverse effect of preoperative steroid treatment.[17] Our findings suggest the eventual necessity of longer-term administration of corticosteroids. With respect to the aspect of cytokine balance, steroid administration or anti-inflammatory treatment should be required only from postoperative Day 2 up to the end of the first week. Cytokine response after OP surgery, however, does not require any anti-inflammatory treatment because it is balanced with anti-inflammatory batteries.

The inspection of the increased myocardial outflow of different cytokines during CPB is another interesting finding of the present study. In the OP group, no obvious differences could be observed between cytokines of peripheral-venous and CS blood. To our knowledge, this is the first study comparing the myocardial production of cytokines during use of the CPB and OP techniques. Despite cardioprotection with cardioplegia and cooling, the heart is exposed to relatively long-term and global ischemia as a result of cross clamping of the aorta, which may provoke myocardial cytokine production due to large amounts of free radicals resulting from the activation of nuclear factor-kappa B (NF-KB).[18, 19] In the course of OP surgery, however, myocardial and cardiac endothelial cells respond to brief and partial ischemic periods. Brief periods of coronary ligatures during ischemic preconditioning may result in protection via protein production with protective effects and without expression of cytokines.

Numerous papers have recorded significantly elevated IL-6, IL-8, and TNF concentrations of CS compared with arterial blood without deviance of IL-10.[6, 20] Since cytokine levels are elevated in the CS, the local concentration of these cytokines may increase more noticeably during and after CPB, causing augmented reactions and injurious effects in the reperfused heart.

Limitations

The relatively small number of patients investigated may be a limitation of the present study. A larger number of patients would allow for the examination of cytokine balance and myocardial cytokine production in high-risk patients or in patients with prolonged recovery, postoperative myocardial dysfunction, respiratory failure, and so forth. The fact that the anesthetic technique was not entirely the same in the groups may also be a limitation. Using different techniques made it difficult to standardize anesthesia and intraoperative treatment. Studies investigating the effect of anesthesia on cytokine production state indicate a change in selected cytokines after anesthesia with different agents. Most of these studies applied < 24 h observation periods and examined cytokines that changed just after wound closure as a direct effect of chosen anesthetic management.[21]

Conclusion

The present study has attempted to give a comprehensive view of the alteration of the cytokine network during and after CABG with and without CPB using a novel method, the CBA technique. The investigations highlight the usefulness of this technique, also in clinical practice, for obtaining pro- and anti-inflammatory response because it is reliable and simple. Hence, in the course of CPB, the elevation of proinflammatory cytokine is highly counterbalanced by the systematic release of IL-10 during and in the early period following the surgery. Consequently, after postoperative Day 1, a significantly elevated pro-/anti-inflammatory cytokine ratio was measured. In contrast, OP surgery is associated with a rather balanced relation of pro- and anti-inflammatory responses. The concentration of IL-12 was also higher following CPB. This study revealed the myocardial outflow of certain proinflammatory cytokines during CPB, as manifested by a higher sinus level of IL-1, IL-6, IL-8, and TNF, while it was undetected in the course of OP surgery. These results may reflect the different cellular effects of the two procedures and aim to improve our understanding of the impact of CPB on patients.

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