IL-8 concentration in coronary sinus blood during early coronary reperfusion after ischemic arrest

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Abstract

Objective: Activation of the inflammatory response is an important factor contributing to complications of cardiopulmonary bypass. Increased level of proinflammatory cytokine – IL-8 has been reported during coronary artery bypass grafting (CABG) operations with the use of cardiopulmonary bypass. The aim of this study was to find out whether the heart is the main source of IL-8 during early coronary reperfusion. Methods: IL-8 concentration in coronary sinus before clamping and 5, 10, and 15 min after declamping of the aorta as well as in radial artery blood before clamping and 10 min after declamping of the aorta, was assessed in 30 patients undergoing CABG surgery. Results: We observed increase in IL-8 concentration in coronary sinus blood after declamping of the aorta, however no difference between coronary sinus and arterial blood concentration was noted. The median value of IL-8 concentration in coronary sinus blood was 1.85 pg/ml before ischemia and 15.4, 20.3, and 29.3 pg/ml in 5, 10 and 15 min after aortic declamping, respectively. Our additional finding was that there was a negative correlation between IL-8 level and hemoglobin saturation with oxygen in coronary sinus blood 10 min after coronary reperfusion. Conclusions: We conclude that the heart is not the main source of IL-8 in early coronary reperfusion, although coronary reperfusion induces its release. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cardiopulmonary bypass (CPB) is inevitable for performing many cardiac operations. Activation of the immune system during CPB is one of its most clinically relevant disadvantages. Proinflammatory cytokines play a key role in the inflammatory cascade induced during CPB with an increase in body temperature and interstitial protein reach edema being the most common manifestations. In recent years numerous studies on inflammatory response to CPB have widened our understanding of this phenomenon. It consists of several factors, including activation of the complement system from the beginning of the CPB [1], release of endotoxin during and after the CPB, and ischemia-reperfusion injury mainly to the heart and lungs [2]. Identifying a contribution of different organs to the cytokine production related to CPB and ischemia-reperfusion can provide us with a better insight into the pathology of inflammatory response to cardiac surgery. IL-8 plays an important role in ischemia-reperfusion injury after the CPB [3]. Its release is probably not stimulated directly by contact between the blood and extracorporeal circuit. Ivey and coworkers postulated that IL-8 generation during reperfusion of the ischemic myocardium might be indirectly dependent on production of complement C5a fragment [4]. Activation of the local myocardial gene expression for IL-8 messenger RNA was reported during pediatric CPB [5]. IL-8 was reported to be lower in patients undergoing off-pump revascularisation than in patients submitted to classical coronary artery bypass grafting (CABG) [6]. It has been reported from an experimental study that IL-8 is released during reperfusion of the ischemic myocardium [4,7]. Cardiac release of IL-8 was observed after myocardial infarction treatment with percutaneous transluminal coronary angioplasty [8]. Other organs, especially the liver and the lungs, have also a potential for IL-8 release during reperfusion after ischemia [9]. It was observed that jugulo-arterial IL-8 gradient after CPB was especially pronounced after normothermic CPB. It suggests that the brain is an important source of IL-8 after CPB [10]. Ischemia-reperfusion in the pulmonary vascular bed has been also postulated to be an important source of IL-8 after CPB.
A negative inotropic activity of cytokines has been described. They are claimed to have an important role in myocardial stunning [11]. A peripheral vasodilation seen in response to CPB may be the manifestation of cytokine-mediated nitric oxide synthase induction in smooth muscle of vascular wall [3].

IL-8 may be produced by a variety of different cells including neutrophils, monocytes, lymphocytes-T, and endothelium. It is a potent chemotactic factor that promotes transendothelial neutrophil migration [12]. Furthermore it activates neutrophils for a respiratory burst, induces their degranulation and thus a damage to the endothelial basement membrane [12].

Activated neutrophils release reactive oxygen radicals, cationic proteins and elastase, which, together with other proteolytic enzymes, cause endothelial injury and can induce tissue damage manifested as a multiple organ dysfunction. The administration of IL-8 resulted in pulmonary accumulation of neutrophils and tissue injury in experimental study [13].

Assuming prevalently negative effect of inflammatory reaction to CPB on morbidity, many attempts has been made in order to reduce its negative influence on the clinical outcome [6]. Among them administration of steroids was reported to be effective in decreasing TNF-α, IL-6 and IL-8 levels [14,15].

The purpose of the present study was to define whether the heart is the main source of IL-8 in the first minutes of reperfusion after aortic cross-clamp release and what are other factors responsible for changes of IL-8 level in blood.

2. Materials and methods

Thirty consecutive patients (24 men and six women) undergoing coronary artery bypass grafting (CABG) with the use of CPB, with indications for retrograde cardioplegia administration, were included into the prospective study. Left main stenosis or proximal LAD with circumflex artery stenosis or left ventricular hypertrophy was considered an indication for administration of retrograde cardioplegia. The exclusion criteria were: an inflammatory process or infection within two weeks before the operation, myocardial infarction during the last three months before the operation, and the age of more than 70 years. Four of our patients had diabetes mellitus. Informed consent was obtained from every patient included in the study protocol. The study has been approved by the institutional ethics committee on human research in the Medical University of Gdansk. Anesthesia was performed with fentanyl 20–40 μg/kg (Fentanyl, Polfa, Warsaw, Poland), and either metohexitol (Brietal, Eli Lilly, USA), or midazolam (Dormicum, Hoffman – La Roche, Basel, Switzerland). Muscle relaxation was achieved with pancuronium (Pavulon, Organon Teknika, Boxtel, The Netherlands) 0.08 mg per kg of body weight. Cefazoline (Kefzol, Eli Lilly, Florence, Italy) 1 g every 6 h for 24 h was administered intravenously as an infection prophylaxis. 15 patients received 1 mg/kg body weight of dexamethasone (Dexaven, Jelfa, Jelenia Gora, Poland) prior to the CPB. Anticoagulation for CPB was induced with 3 mg/kg of heparin. Further doses were administered if necessary to maintain the activated clotting time of more than 450 s. A catheter for administration of retrograde cardioplegia was inserted into coronary sinus directly after beginning of the CPB. After weaning from CPB heparin was neutralized with 1.2 mg of protamine sulfate for each mg of heparin. The extracorporeal circuit consisted of a roller pump COBE Perfusion System (COBE, Arvada, CO, USA) and a membrane oxygenator Compactflo System 0703 (Dideco, Mirandola, Italy). During normothermia a pump flow was maintained at 2.4 l/m²/min. Five hundred millilitres of St. Thomas cardioplegic solution was administered as well ante- as retrograde for myocardial protection against ischemia. All patients were cooled to 32°C. Rewarming was started during accomplishing the last but one distal anastomosis. The proximal anastomoses were made with the aid of a tangential clamp after aortic cross-clamp release. Samples of arterial and sinus coronary blood were collected simultaneously after insertion of the catheter for retrograde cardioplegia (before aortic cross-clamping - point 1) and 10 min after the aortic cross-clamp had been removed (point 3). Another blood sample from coronary sinus was drawn 5 (point 2) after aortic cross-clamp removal. In patients who required more than 15 min of reperfusion additional blood sample was taken at that time (point 4). A fraction of each blood sample was submitted to blood gas analysis, while the rest of sample was immediately centrifuged at 4000 × g for 5 min and stored at −70°C until the assay for IL-8 was performed. IL-8 concentration was determined with enzyme-linked immunosorbent assay (ELISA) (Endogen Inc. USA). The sensitivity of this assay, as defined by manufacturer, is 2 pg/ml.

Changes of IL-8 concentration were assessed with Wilcoxon signed rank test with Bonferroni’s correction. Ten minutes after aortic cross-clamp removal (point 3) we compared IL-8 concentration in coronary sinus blood between diabetic and diabetes-free patients, and in patients who received dexamethasone 1 mg/kg with patients given no dexamethasone. At point 3 IL-8 concentration was compared between arterial and coronary sinus blood in all patients. Above-mentioned analysis was tested with Mann-Whitney U test. Spearman’s test was performed for correlation of IL-8 concentration with saturation of hemoglobin with oxygen and the aortic cross-clamp time. Statistical significance was attributed to P value lower than 0.05.

3. Results

The clinical data of the studied patients are summarized in Table 1.

During early coronary reperfusion we observed an
increase in IL-8 concentration in coronary sinus blood. Differences between IL-8 concentration in particular points in time were statistically significant. IL-8 levels before cross-clamping of the aorta, 5, 10, and 15 min after removal of aortic cross-clamp are presented in Fig. 1. Blood samples collected 15 min after aortic cross-clamp release, were obtained only in 12 out of 30 patients. In 18 patients coronary sinus line had been removed before that time.

Median value of IL-8 in coronary sinus blood 10 min after release of cross-clamp was 20.3 pg/ml (Q1 = 13.6, Q3 = 37.2, range: 0–108.7) and in radial artery blood was 24.6 pg/ml (Q1 = 11, Q3 = 33, range: 0–66). The difference observed between IL-8 concentration in blood in coronary sinus and radial artery 10 min after aortic declamping was not statistically significant (P = 0.65).

The median value of IL-8 in coronary sinus blood 10 min after release of cross-clamp was 20.3 pg/ml (Q1 = 13.6, Q3 = 37.2, range: 0–108.7) and in radial artery blood was 24.6 pg/ml (Q1 = 11, Q3 = 33, range: 0–66). The difference observed between IL-8 concentration in blood in coronary sinus and radial artery 10 min after aortic declamping was not statistically significant (P = 0.65).

IL-8 concentration in coronary sinus blood of diabetic patients (Median = 15.6 pg/ml, Q1 = 3.8, Q3 = 43.6, range: 0–52.8), was not statistically different from that of diabetes-free patients (Median 21.3 pg/ml, Q1 = 13.4, Q3 = 37.3, range: 1.9–108.7) (P = 0.5).

We found a negative correlation between IL-8 concentration in coronary sinus blood and saturation of hemoglobin with O₂ in coronary sinus blood (Fig. 2).

In our patients we observed no correlation between IL-8 level and the aortic cross-clamp time.

4. Discussion

The main purpose of the study was to assess the influence of myocardial ischemia-reperfusion on IL-8 release. A step increase in IL-8 concentration in coronary sinus blood was observed after release of aortic cross-clamp. We did not assess IL-8 concentration before induction of the anesthesia and surgery. It has been reported previously that intravenous anesthesia does not induce IL-8 release and that IL-8 release is not dependent from the doses of fentanyl [16,17]. On the other hand IL-8 release is known being induced by general surgery, with the peak level on days 1 and 3 after the operation [18]. The median of baseline value of IL-8 in our patients was 1.95 pg/ml which is within the range of normal plasma concentration defined by the assay manufacturer (0 to 8.1 pg/ml). We assume that IL-8 release induced by a surgical trauma is characterized with certain delay that was responsible for the fact that our baseline level was within the physiologic range in spite of anesthesia and surgery.

Obviously we could not assess IL-8 concentration in the coronary sinus blood directly before aortic cross-clamp removal. However, a distinct increase of its concentration in coronary sinus blood indicates that the restoration of the coronary blood flow after release of the aortic cross-clamp acts as a trigger for increase of IL-8 concentration in the blood. After 15 min of reperfusion blood samples were taken from coronary sinus only in 12 out of 30 patients. This reduction was due to the fact that 18 patients did not require longer reperfusion and cardiopulmonary bypass was
about to be finished before 15 min after aortic cross-clamp removal. The blood samples from coronary sinus were collected through a catheter for retrograde cardioplegia, which had to be removed before the end of cardiopulmonary bypass.

We followed IL-8 level in a very short time after the CPB that covered only the very beginning of its release after myocardial reperfusion. It is known from other studies, that the pattern of IL-8 level after the CPB has a bimodal distribution, peaking about 5 h after myocardial reperfusion, and again about 16–18 h after the operation [2].

Lack of difference in IL-8 concentration between sinus coronary and arterial blood may indicate that also other than coronary vascular bed is an important place of its release. On the other hand an increase in IL-8 concentration in the first few minutes after aortic declamping, strongly suggests that during coronary reperfusion a substance that stimulates IL-8 release, may be washed out from coronary vascular bed. This substance could be TNF-α, which has the potential for stimulating IL-8 production and is released from coronary circulation in a similar time-profile [19]. As it has been proven previously, the delay that exists between the first peak of TNF-α and the first peak of IL-6 and IL-8 is consistent with the function of TNF-α as an initiator for the cytokine cascade [2,19]. This hypothesis can be supported by the results of Marx, who observed that plasma from the ischemic and reperfused heart stimulates the expression of IL-1β and IL-8 in leukocytes [20].

It has been reported that IL-8 release in response to cardiopulmonary bypass is much more pronounced in the lungs and pulmonary vascular bed, than in monocytes in systemic vascular bed [21]. In our patients IL-8 concentration in the arterial blood was slightly but not significantly higher than in coronary sinus blood, so that we can not exclude that the lungs are its important source.

It can be speculated that all possible sites and mechanisms of IL-8 generation, including release from circulating monocytes and neutrophils, are active during CPB, which makes difficult to determine its main source.

We noted no difference in IL-8 levels between diabetic and diabetes-free patients. Our data are not consistent with observations by Nawas et al. who noted elevated sinus coronary IL-8 after reperfusion only in diabetic patients [22].

Corticosteroids have been administered to patients submitted to CPB for many years, in order to reduce activation of the inflammatory response. Its administration before CPB has been found to reduce complement activation [23]. Engelman and coworkers found decrease in complement activation as well as in IL-1β and IL-8 concentration in patients primed with 1 g of methylprednisolone [14].

Tassani et al. observed efficient decrease of IL-8 concentration by methylprednisolone administration during aprotinin treatment in patients operated with the use of CPB [24].

Our results did not confirm that the early IL-8 release is suppressed by steroids pretreatment before CPB, but we can not exclude that inhibitory effect of steroids on IL-8 release can be observed later in time.

In our study a negative correlation has been found between IL-8 concentration in coronary sinus and hemoglobin saturation with oxygen in coronary sinus blood, suggesting that the higher oxygen extraction in coronary vascular bed, the higher IL-8 release. Previously a time-dependent generation of IL-8 has been observed in human endothelial cells culture submitted to ischemia [25]. The negative correlation between IL-8 and hemoglobin saturation with oxygen in coronary sinus can be explained either by the direct IL-8 release from ischemic endothelium, or by the indirect stimulation of IL-8 release mediated by other substance.

In our patients measurement of the IL-8 concentration in a very short time after the aortic cross-clamp release could not detect correlation with the time of myocardial ischemia, which was reported by some investigators [2,12]. Our observation is consistent with Ferring and associates, who did not observe any correlation between CPB time and aortic cross-clamping time [7].

In the literature a great variability among the results of studies on IL-8 is observed. It is attributed to binding of IL-8 to plasma proteins, assay inhibition by specific proteins, rapid interleukins degradation by plasma proteases, rapid clearance from the circulation by cell receptors, and transiently elevated levels that are easily missed [2].

Improved understanding of the inflammatory reaction may contribute to increased safety of the patients submitted to CPB, and possibly can lead to development of the treatment aimed at reduction of CPB complications.

5. Conclusions

On the basis of this study we conclude that:

1. Heart is not the main source of IL-8 in early coronary reperfusion during classical CABG operation, although coronary reperfusion induces the IL-8 release.
2. IL-8 concentration in coronary sinus blood after aortic cross-clamp removal negatively correlates with hemoglobin O₂-saturation in coronary sinus blood.

References


