The effect of methylprednisolone treatment on the cardiopulmonary bypass-induced systemic inflammatory response

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Abstract

Objective: Cardiac surgery with cardiopulmonary bypass (CPB) is associated with an inflammatory response caused by contact of blood with artificial surfaces of the extracorporeal circuit, ischemia-reperfusion injury, and release of endotoxin. The inflammatory reaction involves activation of complement leucocytes, and endothelial cells with secretion of cytokines, proteases, arachidonic acid metabolites, and generation of oxygen derived free radicals (OFR) by polymorphonuclear neutrophils (PMN). Although this inflammatory response to CPB often remains at subclinical levels, it can also lead to major organ dysfunction. A number of studies have demonstrated that treatment of patients with a high-dose (30 mg/kg) of corticosteroids (methylprednisolone) attenuates the CPB-induced SIR and improves the outcome of patients undergoing cardiac surgery. However, large doses of steroids can cause abnormal metabolic responses such as metabolic acidosis and hyperglycemia. In the present study, we examined the efficacy of low doses of methylprednisolone (5 and 10 mg/kg) to attenuate the CPB-induced inflammatory response, during and after heart operations.

Methods: Thirty-six adult patients undergoing cardiac surgery, were randomized into three groups: (1) control group: group A; (2) methylprednisolone, 5 mg/kg body weight: group B; and (3) methylprednisolone, 10 mg/kg body weight: group C. Plasma levels of the cytokines interleukin-6 (IL-6) and TNF-α were analyzed by enzyme-linked immunosorbent assay, before, during, and after CPB. OFR production was determined by cytofluorometry (FACS) at the same end points.

Results: No significant differences in age, body weight, CPB time, and cross-clamp time were observed among the three groups. CPB induced a marked increased in cytokine release and OFR generation. Low-dose of methylprednisolone (5 mg/kg) effectively reduced the increase in TNF-α and IL-6 secretion (P<0.05 compared to control group) after release of the cross-clamp. However, OFR generation was significantly reduced with a greater dose of methylprednisolone (10 mg/kg).

Conclusions: The results indicate that a single low-dose of methylprednisolone (10 mg/kg) reduces the inflammatory reaction during and after CPB, by inhibition of proinflammatory cytokine release and OFR generation after release of the aortic cross-clamp.

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1. Introduction

Cardiac surgery is a well-accepted treatment for ischemic, valvular or congenital heart diseases, with low morbi-mortality rate. Although CPB is fundamental for most cardiac operations, it is associated with a complex systemic inflammatory response (SIR) [1]. The SIR in cardiac surgery is mainly caused by contact of blood with the artificial surface of the extracorporeal circuit, ischemia-reperfusion injury, changes in body temperature, release of endotoxin [2] and operative trauma.

CPB has been shown to induce complement activation, release of cytokines, leucocytes activation, the expression of adhesion molecules, and the release of many inflammatory mediators including oxygen free radicals, arachidonic acid metabolites, cytokines, platelet activating factor, nitric oxide, and endothelins. This inflammatory cascade can lead to post-operative complications such as respiratory failure,
renal dysfunction, altered liver function, and ultimately, multiple organ failure [1].

However, the exact mechanisms of this inflammatory response to CPB remain to be fully determined.

CPB activates the complement system leading to granulocyte activation [3], which in turn produce and release oxygen free radicals (OFR), including superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen. These OFR act on membrane lipids to increase membrane permeability, and may also decrease cardiac and pulmonary function [4].

Considerable interest has been recently focused on the involvement of cytokine network during and after CPB.

Next to free radical production, CPB also induces a systemic cytokine release [5]. The release of cytokines during CPB can have deleterious effects on the heart and other organs. Proinflammatory cytokines (TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-8 (IL-8)) can significantly alter myocardial contractility, peripheral circulation and also exert a direct damaging effect on the other organs [6].

The release of these proinflammatory cytokines can be stimulated by a number of factors including ischemia-reperfusion, complement activation, endotoxin release and the effect of other cytokines [7]. Increased levels of TNF-α and IL-6 during and after CPB have been shown by many studies [7].

The CPB-induced SIR is multifactorial, but is undoubtedly that release of proinflammatory cytokines and production of OFR are important events of this complex inflammatory reaction.

We decide to measure TNF-α and IL-6 as a reflect of CPB-induced SIR. Experimental data indicate that TNF-α is a major proinflammatory cytokine known to be produced in excessive amounts after CPB, and is one of the earliest endogenous mediators released as a result of the inflammatory response.

IL-6 was measured because is a multifunctional cytokine, generally considered a non-specific marker of inflammation, that regulate immune responses, and is associated with the diffuse inflammatory response after CPB.

Great effort have been focused on therapeutic interventions to reduce the inflammatory reactions during CPB.

The aim of the present article is the evaluation of steroid pre-treatment as a possible strategy to inhibit the inflammatory response associated with CPB.

A number of studies have demonstrated that treatment of patients with a large dose (30 mg/kg) of corticosteroids (methylprednisolone) attenuates the CPB-induced SIR in patients undergoing cardiac surgery. The steroid treatment is reported to reduce the complement-mediated activation of neutrophils [8] and inhibit the secretion of proinflammatory cytokines, including TNF-α [9] and IL-6 [10]. However, the influence of corticosteroids on the inflammatory response and on the post-operative course is still controversial. For example, a recent CABG study found that a high-dose of methylprednisolone (30 mg/kg × 2) was associated with an increase in post-operative alveolar-arterial oxygen gradient and shunt and a prolonged time of tracheal intubation [11].

The dose of corticosteroids commonly used during CPB was derived from septic shock studies which demonstrated that at least 30 mg/kg of methylprednisolone was required to demonstrate an effect on patient survival [12]. Although CPB complications and septic shock are both related to SIR, significant difference exist that could explain why the use of high-dose corticosteroid may not be appropriate in the former.

A review of the literature did not reveal any dose–response type studies conducted on CPB patients to determine the optimal dose required to attenuate the CPB-induced SIR [13].

Therefore, we initiated a prospective, randomized, double blinded study to compare the efficacy of two low doses of methylprednisolone (5 and 10 mg/kg) to attenuate the CPB-induced SIR. We showed that 10 mg/kg dose was high enough to significantly reduce the cardiac surgery associated SIR on biological parameters.

2. Methods

2.1. Patients

Thirty-six adult patients undergoing elective coronary artery bypass grafting procedures were studied in a randomized trial. The study was approved by the local medical ethical committee. All patients gave their informed consent. Exclusion criteria were redo operation, age under 50 or over 80, weight under 60 or over 90 kg, renal or hepatic dysfunction, hematologic or coagulation disorders, infection during the week preceding surgery, pre-operative use of antibiotics or corticosteroids and white blood cell count over 11,000 mm−3. Patients were randomized equally to one of the three following groups: group A, control group, no treatment; group B, methylprednisolone 5 mg/kg; group C, methylprednisolone 10 mg/kg. Methylprednisolone was infused just at the beginning of the CPB.

2.2. Technique of the CPB

The extracorporeal circuit consisted of a hollow-fiber membrane oxygenator, with integrated cardiotomy filter (Monolyth, Sorin Biomedica cardio, Saluggia, VC, Italy), an extracorporeal circuit line set (Sorin Biomedica cardio, Saluggia, VC, Italy), and a roller pump. Heparin (3 mg/kg body weight) was given in the right atrium before cannulation of the aorta. Reinfusion of 25 mg of heparin was repeated whenever the activated clotted time was shorter than 400 s. Temperature core was reduced to
28–32 °C and a non-pulsatile flow of 2.4 l/min per m² was maintained. Myocardial protection was achieved by antegrade administration of 1 l of crystalloid solution at 4 °C, in a single dose. At the termination of the CPB, heparin was neutralized with protamine sulfate using 60% of the heparin.

2.3. SIR assessment

Blood samples were taken from the arterial line at the following time-points: (1) after anesthesia induction; (2) before aortic cannulation; (3) 10 min after aortic cross-clamp release; and (4)–(6) 5 min, 4 and 24 h after protamine sulfate administration, respectively. Blood samples were immediately brought to the laboratory and processed. Before, during and after the CPB, blood samples were taken for analysis of blood gas, white blood cells and platelets counts, hematocrit, hemoglobin, electrolytes (Na⁺, K⁺, Ca²⁺), BUN, creatinine, serum glucose and lactates levels.

2.4. Flow cytometry (OFR)

Blood was drawn in heparin lithium containing tubes and processed within the 2 h. One-milliliter of blood was gently mixed with 1 ml of the nuclear dye LDS-751 (1 μg/ml; Molecular Probes®). LDS-751 stains specifically nucleated cells and fluoresces in the far red region, allowing us to analyze by flow cytometry nucleated cells in whole blood, without getting background signal from red cells. Aliquots of the whole blood/LDS-751 mixture (250 μl) were then distributed in three tubes. Samples were then incubated either with PBS (negative control) or with 118 μl of 2'/7' dichlorofluorescein diacetate (DCFH-DA, 80 mmol/l; Molecular Probes®) for 15 min in a 37 °C waterbath. DCFH-DA is a fluorescent probe used to measure intracellular OFR [14] After 15 min, the standard neutrophil agonist N-formyl-L-methionyl-L-leucyl phenylalanine (fMLP, SIGMA®) was added (final concentration 10⁻⁷ mol/l) in one of the DCFH-DA tube (positive control), and incubation was continued for an additional 10 min. At the end of the incubation time, chemical reactions were stopped by adding 4 ml of cold PBS in each tube. Tubes were stored in ice until data acquisition by flow cytometry analysis [15].

Leucocyte generation of OFR was measured using a FACScan flow cytometer (Becton Dickinson). A 488-nm argon laser light was used for excitation, and fluorescence emission was detected as forward scatter (FSC), which is a measure of cell size, and side scatter (SSC), which is a measure of cell granularity. Fluorescence intensity was detected in channel 1 (DCFH-DA) and channel 3 (LDS-751). A threshold fluorescence was set on the LDS-751 signal allowing data collection on polymorphonuclear neutrophils (PMN) without interference with erythrocytes. During acquisition, PMN were differentiated from lymphocytes and monocytes on the basis of different forward and SSC plots. Acquisition was performed on 5000 PMN.

During analysis, the result from the negative control was used to set up the M1 marker in order to eliminate background fluorescence (Fig. 1). The positive control (fMLP) was used to note the level of fluorescence that could be achieved when the blood was markedly activated. The results were expressed as the total fluorescence intensity (TFI), which was the product of mean channel fluorescence and percent positive events of the fluorescent probes.

2.5. Cytokine assay

The blood anti-coagulated with ethylenediaminetetraacetic acid was centrifuged at 3000 rpm/min for 10 min and the plasma was frozen and stored at −20 °C for IL-6 and TNF assays. The levels of plasma TNF-α and IL-6 were measured by enzyme-linked immunosorbent assay kits.

Fig. 1. Representative OFR fluorescence histograms from PBS (top), patient sample (middle), and the positive control fMLP (bottom). The rightward shift in the mean channel fluorescence for the patient sample indicates a higher generation of OFR by PMN. Result from PBS control was used to set up a marker (M1) in order to eliminate background fluorescence.
(Immunotech SA, Marseilles, France). The limit of sensitivity of each assay was: TNF-α = 5 pg/ml, IL-6 = 3 pg/ml.

2.6. Statistical analysis

Data are presented as the mean ± the standard deviation (SD) and analyzed by Mann–Whitney U-tests. A P-value of < 0.05 was considered significant.

3. Results

The clinical characteristics of the 36 patients undergoing CPB are summarized in Table 1. No significant differences in age, operation, body weight, and number of diabetic patients were observed between the three groups.

CPB aortic, cross-clamp time and intubation time were similar between groups.

Steroid administration did not induce differences in mechanical ventilation requirement (group A: 12.7 h; group B: 10.8 h; group C: 11.6 h).

There were no operative deaths or important adverse complications.

3.1. SIR assessment

Post-induction baseline levels of IL-6, TNF-α, and OFR was similar in the three groups.

3.1.1. Tumor Necrosis Factor-α

In groups A and B, TNF-α plasma level started to increase before aortic clamping and reached a peak 4 h after protamine sulfate administration. In group C, TNF-α remained significantly low throughout the surgical procedure. Twenty-four hours after protamine infusion, TNF-α was still significantly greater in group A (P < 0.05), whereas it dropped back down to baseline level in groups B and C (Fig. 2).

3.1.2. IL-6

Similar to TNF-α level, IL-6 reached a peak 4 h after protamine infusion. However, in contrast to TNF-α, the peak of IL-6 in group A was significantly higher than in group B (P = 0.0017). In group C, IL-6 level remained significantly less than groups A and B throughout the immediate post bypass period. At the 24 h time-point, IL-6 level in groups B and C was somewhat greater but not significantly different in comparison to the baseline. In contrast, in group A, IL-6 remained significantly elevated (Fig. 3).

3.1.3. OFR

In the three groups, the peak of OFR production occurred earlier than for TNF-α and IL-6 (i.e. after aortic cross-clamp release). At that specific time-point, OFR production was significantly lower in group C (P = 0.02). At the 24 h time-point, OFR levels returned to the baseline pre-operative levels in groups A, B and C, with no significant difference between the 3 groups (Fig. 4).

<table>
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<tr>
<th>Table 1 Characteristics of the patients for groups A, B, and C</th>
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<tr>
<td><strong>Group</strong></td>
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<td>Number of patients</td>
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<td>Age (years)</td>
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<td>Weight (kg)</td>
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<td>CPB (min)</td>
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<td>Cross-clamp time (min)</td>
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<td>Intubation time (h)</td>
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<td>Diabetes mellitus</td>
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Values given as mean ± SD. No significant differences were found between groups in any variable measured. CPB, cardiopulmonary bypass.

Fig. 2. Plasma TNF-α of patients treated with methylprednisolone 5 mg/kg (square), 10 mg/kg (circles) or placebo (triangles). Time points: PI, after anesthesia induction; BC, before aortic cannulation; PD, 10 min after aortic cross-clamp release; PP, 4 h PP, and 24 h PP refers to 5 min, 4 and 24 h after protamine sulfate administration. A significant decrease in TNF-α levels was observed 5 min, 4 and 24 h after protamine sulfate administration in group C (P < 0.05). In group B, the decrease in TNF-α levels was only observed 24 h after protamine sulfate administration (P < 0.05).

Fig. 3. Graph illustrating the changes in plasma IL-6 in group A (square), B (circles), and C (circles). Time points: PI, after anesthesia induction; BC, before aortic cannulation; PD, 10 min after aortic cross-clamp release; PP, 4 h PP, and 24 h PP refers to 5 min, 4 and 24 h after protamine sulfate administration. A decrease in IL-6 levels was observed in groups B and C compared to group A (P < 0.01) 4 and 24 h after protamine sulfate administration. Five minutes after protamine administration a significant decrease is only observed in group C (P < 0.01).
Fig. 4. OFR generation (during and after CPB) is measured by monitoring DCFH-DA fluorescence in PMN by FACS analysis. TFI is the product of mean channel fluorescence and the percentage of positive cells, in group A (square), B (circles), and C (triangles). Time points: PI, after anesthesia induction; BC, before aortic cannulation; PD, 10 min after aortic cross-clamp release; PP, 4 h PP, and 24 h PP refers to 5 min, 4 and 24 h after protamine sulfate administration. The baseline OFR generation rose progressively during CPB, reaching a peak after aortic declamping in the group A. OFR production was significantly decreased after aortic declamping in group C ($P < 0.005$). Following 24 h after CPB the OFR levels returned to the baseline pre-operative levels in groups A, B and C with no significant differences between the three groups.

4. Discussion

The principal finding of this study was that a single, pre-operative, low-dose (10 mg/kg) of methylprednisolone reduced the CPB-induced SIR, as assessed by PMN OFR generation, and plasma TNF-$\alpha$ and IL-6.

4.1. CPB and SIR

CPB is known to induce complement and leukocyte activation as well as the release of inflammatory mediators such as OFR and cytokines [4,5]. These mediators contribute, directly or indirectly, to the occurrence of cardiac and pulmonary dysfunction, following cardiac surgery [8]. In our study, group A patients (control group) demonstrated an early increase in TNF-$\alpha$ concentration (i.e. before aortic cross-clamp time) followed by a peak 4 h after protamine infusion. Moreover, TNF-$\alpha$ remained elevated 24 h after protamine infusion. TNF-$\alpha$ is released by activated monocytes and macrophages and is one of the primary mediators involved in septic shock. TNF-$\alpha$ is known to induce fever, tachycardia, and hypotension, and it also increases microvascular permeability. The release of TNF-$\alpha$ can be stimulated by ischemia-reperfusion, complement activation and endothoxin release during CPB, contributing to the development of multiple organ failure and myocardial dysfunction [16].

IL-6 plasma level started to increase during CPB and peaked 4 h after protamine infusion. Previous studies reported a similar pattern for IL-6 and TNF-$\alpha$ evolution during CPB surgery [17]. This suggests that the reperfusion of heart and lungs rather than CPB itself is the main trigger for SIR. Indeed, PMN are known to be trapped in the coronary and the pulmonary vascular bed during cardiac arrest associated with heart procedures [18]. Moreover, blood stagnation can facilitate PMN adhesion and activation. After aortic cross-clamp release, activated PMN sequester in organs throughout the systemic circulation, releasing proinflammatory cytokines which could, in turn, cause a delayed SIR.

The relationship between CPB surgery and OFR production is more controversial. Several previous studies have demonstrated elevated toxic oxygen metabolites during and after CPB [19]. By contrast other investigators did not find evidence for OFR generation in patients undergoing coronary artery bypass grafting [20]. The limiting factor of these previous studies is that OFR were assessed through an indirect method using malondialdehyde, a lipid peroxidation bioproduct, as a marker of OFR activity. Furthermore, this method is performed after PMN isolation (sedimentation and red blood cell lysis) which is known to spontaneously activate PMN [21] and lead to an increase in OFR production.

Our study is unique in that we assessed OFR production directly in whole blood.

This method utilizes the properties of 2$^{7'}$-dichlorofluorescein diacetate (DCFH), which rapidly diffuses across cell membranes and is then trapped within the cell by a deacetylation reaction. In the presence of hydrogen peroxide and other cellular peroxidases, this compound is oxidized to 2$^{7'}$-dichlorofluorescein (DCF), which is highly fluorescent.

Another important feature of this method is the use of a specific fluorescent dye (LDS-751) that preferentially stains nucleated cells, eliminating erythrocytes from analysis without physical separation of erythrocytes in the sample.

Throughout the procedure, blood was never centrifuged or vortexed but gently mixed with reactants. This allowed us to limit blood activation to avoid a false positive increase in OFR production.

Analysis of intra-PMN OFR production in whole blood requires to isolate PMN with the flow cytometer. This was done by staining the samples with LDS-751. During acquisition, the flow cytometer was set-up with a high-level threshold for red fluorescence in order to only detect white blood cells. The three white blood cell populations (i.e. monocytes, lymphocytes and PMN) were easily distinguished on the basis of different side and FSC plots. Then, fluorescence (530 nm) related to the oxidation of DCFH by OFR was assessed only in PMN population.

This technique enables the direct detection of OFR, based on the oxidation of DCFH by OFR, and not as with other test methods, oxygen degradation intermediate products.

FACS analysis of DCFH-DA is a direct method, which enables to measure the total quantity of oxygen-derived free radicals, including superoxide anion and hydrogen peroxide, produced by the cell of interest.

In our study, we found a progressive increase in OFR production in group A during CPB. However, OFR peaked
right after aortic cross-clamp release, which may be due to PMN activation through reperfusion of the heart and lungs. The fact that OFR level was greatest initially during reperfusion can potentially cause an early reperfusion injury. A reduction in the physiological defenses against OFR is the cause of many diseases, such as inflammation, hyperoxia, acute lung injury, and ischemia-reperfusion syndrome. There is also evidence supporting the involvement of OFR in the pathogenesis of circulatory shock.

4.2. Effect of methylprednisolone on the CPB-induced SIR

Previous septic shock studies demonstrated that a high-dose (30 mg/kg) of methylprednisolone could improve patient’s survival [22]. Corticosteroids have been used in cardiac operations for many years but the exact mechanism of action as well as dose/response efficacy and safety are still controversial.

Steroid administration before CPB has been found to reduce complement activation [9], prevent cytokine release [10], and limit post-operative complications. However, large doses of steroids can cause abnormal metabolic responses such as metabolic acidosis and hyperglycemia. Thus, we decided to test the efficacy of lower doses of methylprednisolone (5 and 10 mg/kg) in order to attenuate SIR with less risk of generating abnormal metabolic response.

Methylprednisolone at 10 mg/kg dramatically inhibited TNF-α production in group C patients. This result is in accordance with the findings of other investigators demonstrating inhibition by dexamethasone of TNF-α released in the circulation after reperfusion of the heart and the lung [23]. Teoh et al. [9] also reported that steroid administration before CPB can reduce TNF-α production after heart surgery. Other studies have demonstrated that corticosteroids down-regulate TNF-α production by inhibiting TNF-α gene transcription.

Although the TNF-α levels were similar in groups A and B during and after the surgical procedure, the level was significantly lower in group B 24 h after protamine infusion. At that time-point, levels were comparable in groups B and C. This result could be explained by the fact that steroids increase the release of the anti-inflammatory cytokine Interleukin-10 (IL-10) [24]. Once secreted, IL-10 can directly inhibit the release of proinflammatory cytokines such as TNF-α, and thus reduce SIR.

In group C, the peak of IL-6 was significantly reduced. This result could be explained by inhibition of TNF-α secretion, which is a potent activator for IL-6 production. However, whereas TNF peak levels were similar in groups A and B, IL-6 peak tended to be lower in group B. This finding suggests another mechanism, other than the exclusive effect of methylprednisolone on TNF-α. The inhibition of IL-6 release after CPB may be explained by blocking of IL-6 gene expression by glucocorticosteroids [25]. Further, the inhibition of IL-6 release may be due to the release of the proinflammatory cytokine IL-10. The IL-6 results are in accordance with results of other clinical studies [10]. Hogevoel et al. observed attenuation of the usual increase in IL-6 in three patients receiving 30 mg/kg of methylprednisolone 90 min before hip surgery. Moreover, Schultz et al. demonstrated that a single high-dose of Methylprednisolone before colonic surgery may reduce the inflammatory response.

Methylprednisolone was also able to significantly reduce the OFR production peak in group C. This inhibition of OFR release clearly demonstrates an inhibitory effect of 10 mg/kg corticosteroids on activated leukocytes. Corticosteroids are known to be suppressive on PMN. PMN recruitment and adhesion are important steps for their activation. Steroids have been shown to inhibit chemotactic peptide binding on PMN’s surface receptors, and down-regulate PMN’s surface receptors.

To our knowledge, this is the first investigation of the effect of a single low-dose of methylprednisolone on the inflammatory response induced by CPB. By quantifying proinflammatory cytokines (TNF-α and IL-6), and free radical generation by PMN throughout CPB surgery we demonstrated that low-dose of methylprednisolone could inhibit SIR. This result should undergo more extensive clinical investigation.

References


