Cell salvage of cardiotomy suction blood improves the balance between pro- and anti-inflammatory cytokines after cardiac surgery

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Abstract

OBJECTIVES: The inflammatory response after cardiac surgery is characterized by a profound release of pro- and anti-inflammatory cytokines. Recent data suggest that the balance between pro- and anti-inflammatory cytokines is of greater importance than the absolute levels. Retransfusion of unwashed cardiotomy suction blood contributes to the inflammatory response, but the balance between pro- and anti-inflammatory cytokines in cardiotomy suction blood and whether cell salvage before retransfusion influences the systemic balance have not been investigated previously.

METHODS: Twenty-five coronary artery bypass grafting patients were randomized to either cell salvage of cardiotomy suction blood or no cell salvage before retransfusion. Plasma levels of three anti-inflammatory cytokines [interleukin (IL)-1 receptor antagonist, IL-4 and IL-10] and two proinflammatory cytokines (tumour necrosis factor-alpha and IL-6), and the IL-6-to-IL-10 ratio was measured in cardiotomy suction blood before and after cell salvage, and in the systemic circulation before, during and after surgery.

RESULTS: Plasma levels of all cytokines except IL-4 and IL-10 were significantly higher in cardiotomy suction blood than in the systemic circulation. The IL-6-to-IL-10 ratio was 6-fold higher in cardiotomy suction blood than in the systemic circulation [median 10.2 (range 1.1–75) vs 1.7 (0.2–24), P < 0.001]. Cell salvage reduced plasma levels of cytokines in cardiotomy suction blood and improved the systemic IL-6-to-IL-10 ratio 24 h after surgery [median 5.2 (3.6–17) vs 12.4 (4.9–31)] compared with no cell salvage (P = 0.032).

CONCLUSIONS: The balance of pro- and anti-inflammatory cytokines in cardiotomy suction blood is unfavourable. Cell salvage reduces the absolute levels of both pro- and anti-inflammatory cytokines in cardiotomy suction blood and improves the balance in the systemic circulation after surgery.

Keywords: Extracorporeal circulation • Cell salvage • Inflammation • Cytokines • Cardiotomy suction

INTRODUCTION

Cardiac surgery induces a systemic inflammatory response that may contribute to postoperative organ dysfunction and complications [1, 2]. A number of factors have been suggested to contribute to the inflammatory response, such as the operative trauma per se, the use of cardiopulmonary bypass, ischaemia/reperfusion injury and endotoxin release [1–3].

The inflammatory response after cardiac surgery is characterized by complement activation and cytokine release [1–3]. Cytokines can have pro- or anti-inflammatory properties, or both. Anti-inflammatory cytokines counteract proinflammatory activation, and they may therefore protect tissues and organs during and after cardiac surgery. Recent data from patients with different inflammatory conditions suggest that the balance between pro- and anti-inflammatory cytokines is at least as important as the absolute levels of cytokines [4–9]. The balance is often presented as the IL-6-to-IL-10 ratio.

Our group and others have demonstrated that retransfusion of unwashed cardiotomy suction blood contributes to the inflammatory response after cardiac surgery [10]. This is not surprising, since it has been shown that cardiotomy suction blood contains markedly elevated levels of proinflammatory cytokines [10–15]. There is limited information available on anti-inflammatory cytokines in cardiotomy suction blood, and it is not known whether the balance between pro- and anti-inflammatory cytokines differs between cardiotomy suction blood and the systemic circulation during on-pump cardiac surgery.

Cell salvage reduces the content of proinflammatory cytokines in cardiotomy suction blood, and retransfusion of salvaged cardiotomy suction blood has also been shown to reduce the systemic concentrations of proinflammatory cytokines after cardiac surgery [11, 13–15]. However, it is not well understood how cell salvage affects the levels of anti-inflammatory cytokines in cardiotomy suction blood, and whether the balance between pro- and anti-inflammatory activation is influenced if cardiotomy suction
blood is processed before transfusion. It is possible that cell-saver processing reduces the amount of anti-inflammatory substances to the same extent as pro-inflammatory cytokines, resulting in a zero net effect.

The aims of this study were to describe the balance between pro- and anti-inflammatory cytokines in cardiomyotomy suction blood and in the systemic circulation during on-pump cardiac surgery, and to determine whether cell salvage of cardiomyotomy suction blood influences the balance. For these purposes, we designed a prospective randomized study.

**MATERIALS AND METHODS**

**Patients**

Twenty-five coronary artery bypass grafting (CABG) patients were included in a prospective open randomized study after informed written consent. There were 80% men, mean age 68 ± 8 years (mean ± standard deviation). The patients were randomized to either cell salvage of cardiomyotomy suction blood before transfusion or no cell salvage. Inclusion criteria were: age 40–80 years, two- or three-vessel coronary disease with angina pectoris and appropriate coronary anatomy for CABG, left ventricular ejection fraction >40% and no other significant disorders. Exclusion criteria were preoperative use of steroids or non steroidal anti-inflammatory drugs (NSAIDs). The patient characteristics are given in Table 1. The Research Ethics Committee of the Medical Faculty, University of Gothenburg, approved the study protocol.

**Clinical management**

All patients were operated on with cardiopulmonary bypass (CPB). The patients were premedicated with flunitrazepam and morphine/scopolamine. Anaesthesia was induced with thiopental 3–5 mg/kg, followed by pancuronium 0.1 mg/kg. Fentanyl was given in incremental doses up to a total amount of 8–10 μg/kg before sternotomy. The patients were normoventilated with oxygen in air (FiO2 0.4–0.5), and enflurane was used as inhalation agent both before and after CPB. Propofol was given during CPB. Before cannulation, heparin (Lövens, Ballerup, Denmark), 300 IU/kg, was given and supplemented as required to maintain an activated clotting time of >480 s. The extracorporeal circuit was primed with approximately 1700 ml Ringer’s acetate (Fresenius-Kabi, Uppsala, Sweden), 200 ml mannitol (Fresenius-Kabi), 100 ml Tribonate (Fresenius-Kabi) and 7500 IU of heparin. CPB was performed with a hollow-fibre membrane oxygenator. A hard-shell reservoir (with blood-air interface) with separate chambers for venous return, and cardiomyotomy suction blood was used (D 903 Avant®, Dideco, Mirandola Modena, Italy). The reservoir design makes it possible to collect cardiomyotomy suction blood and allows blood sampling before the content is returned to the venous reservoir.

The operations were performed with the standard non-pulsatile CPB technique, with moderate hypothermia (nasopharyngeal temperature 34°C) and haemodilution (haematocrit 20–30%). A standard flow of 2.4 l/min/m² was used. Cardioprotection was achieved with cold blood cardioplegia. No topical cooling was used. Weaning off CPB was performed after rewarming to a rectal temperature of at least 36°C. All patients received 2 g of tranexamic acid before surgery and 2 g after protamine was administered. Aprotinin was not used.

**Study protocol and analyses**

The patients were randomly allocated into two groups. In the first group (n = 13), cardiomyotomy suction blood was evacuated from the reservoir and processed in a cell-saver device (autoLog™, Medtronic, Minneapolis, MN, USA) before transfusion. The cell saver produced approximately 135 ml of washed, packed red blood cells. In the second group (n = 12), cardiomyotomy suction blood was collected and retransfused without further processing by opening the hatch between the suction reservoir and the venous reservoir. Ringer’s acetate was added to the processed blood to maintain a retransfusion volume and haematocrit value comparable with those of the unprocessed group.

Concentrations of the anti-inflammatory cytokines interleukin [IL]-1 receptor antagonist (IL-1Ra), IL-4 and IL-10, and the pro-inflammatory cytokines tumour necrosis factor (TNF)-α and IL-6, were measured in the separate cardiomyotomy suction reservoir before and after cell-saver processing and in the systemic circulation (i) at baseline (before surgery), (ii) during CPB immediately before and after cell-saver processing and in the systemic circulation, (iii) before retransfusion of cardiomyotomy suction blood, (iv) before weaning (after retransfusion), (v) 10 min after CPB, (vi) 2 h after CPB and (vii) 24 h after CPB. Haematocrit was measured at all the time points.

The cytokines were assayed with a commercially available enzyme immunooassay (EIA) system (Endogen, Pierce, IL, USA). The detection limits for the different inflammatory variables were as follows: IL-1Ra, 2 pg/ml; IL-4, 2 pg/ml; IL-10, 2 pg/ml; IL-6, 1 pg/ml and TNF-α, 2 pg/ml.

Blood samples for cytokine determinations were drawn into tubes for plasma, containing ethylene diamine tetra-acetic acid (EDTA) added in the proportion of 0.34 M EDTA per 4.5 ml blood. The tubes were centrifuged to remove cells, and the samples were frozen within 30 min, in individual tubes for each determination. They were stored at −80°C until analysis. All assays were done in duplicate.

**Calculations**

Plasma concentrations of the cytokines were adjusted for changes in haematocrit by relating measurements to the preoperative haematocrit: Adjusted concentration = measured concentration ×
(preoperative haematocrit/measured haematocrit). In the statistical calculations, values below the detection limit have been set to 0. The pro-/anti-inflammatory cytokine balance was calculated by dividing the concentration of the proinflammatory cytokine IL-6 by the concentration of anti-inflammatory cytokine IL-10 [5, 9, 10]. It was not possible to calculate the IL-6-to-IL-10 ratio correctly in cardiotomy suction blood after cell-saver processing since IL-10 was completely abolished after processing in some of the plasma samples (division by 0).

Statistics

In all analyses, a P-value <0.05 was considered significant. The results are expressed as median and range or mean and standard deviation. Range and standard deviations are given in parenthesis. The non-parametric Mann–Whitney U-test (continuous non-normally distributed variables), the parametric unpaired t-test (continuous normally distributed variables) and Fisher’s exact test (categorical variables) were used to compare the groups. Differences within a group were compared with either the paired non-parametric Wilcoxon test or the paired t-test when appropriate. Systemic levels of cytokines were compared between the groups with analysis of variance for repeated measurements followed by the t-test at the fixed time points if group analysis or the interaction between group and time analysis indicated a significant difference (P < 0.05). No power analysis for the secondary group comparison was possible since data essential for the analysis (IL-6-to-IL-10 ratio) were unavailable at the start of the study. All statistical analyses were performed with STATISTICA version 10 (StatSoft, Tulsa, OK, USA).

RESULTS

Clinical course

One patient in the cell-saver group developed a perioperative stroke. All other patients were discharged from the hospital within 7 days. No patients received transfusion of blood products during the study.

Baseline variables

There were no statistically significant differences between the cell-saver group and the unprocessed group with respect to age, gender, ejection fraction, aortic clamp time or number of grafts (Table 1).

Cardiotomy suction blood

The median volume of cardiotomy suction blood collected during surgery was 450 ml (225–1100). Plasma concentrations of IL-1r, IL-6 and TNF-α were significantly elevated in unprocessed cardiotomy suction blood compared with systemic plasma concentrations at the same time point, while IL-10 and IL-4 levels were not significantly different (Table 2). The IL-6-to-IL-10 ratio was markedly higher in cardiotomy suction blood than in systemic blood (Table 2). Cell-saver processing of suction blood significantly reduced the plasma concentrations of IL-1Ra, IL-10 and TNF-α (Table 3).

Systemic release of pro- and anti-inflammatory cytokines

TNF-α and IL-6 increased during and after surgery, with peak levels 2 h after surgery. IL-6 levels remained significantly elevated 24 h after surgery, while TNF approached baseline levels at this time point (Table 4). Systemic plasma levels of IL-1Ra and IL-10 increased after the start of the operation and were significantly higher than at baseline in all intraoperative and postoperative measurements (Table 4). IL-4 levels were absent or minimal at all time points. The IL-6-to-IL-10 ratio was elevated at all intraoperative and postoperative measurements relative to the preoperative measurement (Fig. 1).

Effects of cell-saver processing on the systemic balance of pro- and anti-inflammatory cytokines

Patients who had cell-saver processing of cardiotomy suction blood tended to have lower levels of IL-1r, IL-10, TNF-α and IL-6 postoperatively, but the differences did not reach statistical significance (Table 4). The IL-6-to-IL10 ratio was significantly

### Table 2: Cytokines in cardiotomy suction blood and in the systemic circulation at the same time point (n = 25)

<table>
<thead>
<tr>
<th></th>
<th>Cardiotomy suction blood</th>
<th>Systemic circulation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1Ra (pg/ml)</td>
<td>312 (88–1534)</td>
<td>82 (32–309)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>&lt;2 (&lt;2–7.2)</td>
<td>&lt;2 (&lt;2)</td>
<td>0.43</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>197.7 (2–113)</td>
<td>31 (2–122)</td>
<td>0.21</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>210 (13–2678)</td>
<td>45 (3–1119)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>4.3 (&lt;2–40)</td>
<td>2.5 (&lt;2–62)</td>
<td>0.033</td>
</tr>
<tr>
<td>IL-6-to-IL-10 ratio</td>
<td>10.2 (1.1–75)</td>
<td>1.7 (0.2–24)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The values represent the median and range. IL: interleukin; TNF: tumour necrosis factor.

### Table 3: Cytokines in cardiotomy suction blood before and after cell-saver processing (n = 13)

<table>
<thead>
<tr>
<th></th>
<th>Before cell salvage</th>
<th>After cell salvage</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1Ra (pg/ml)</td>
<td>260 (88–526)</td>
<td>73 (28–359)</td>
<td>0.002</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>&lt;2 (&lt;2)</td>
<td>&lt;2 (&lt;2–3.0)</td>
<td>0.18</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>17 (8.4–62)</td>
<td>7 (&lt;2–27)</td>
<td>0.011</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>140 (13–2678)</td>
<td>57 (&lt;1–760)</td>
<td>0.10</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>4.3 (&lt;2–39)</td>
<td>&lt;2 (&lt;2–15)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

The values represent the median and range. IL: interleukin; TNF: tumour necrosis factor.
A complex balance between pro- and anti-inflammatory substances and mediators regulates the human immune system. Cardiac surgery induces an inflammatory response, which may contribute to postoperative organ dysfunction [1–3]. The pro-inflammatory activation is well characterized, while the anti-inflammatory response is less well understood. In the present study, the plasma concentrations of three anti-inflammatory cytokines (IL-1Ra, IL-4 and IL-10) and two pro-inflammatory cytokines (IL-6 and TNF-α) in systemic circulation during and after CABG were investigated in cardiotomy suction blood and in the systemic circulation.

IL-1Ra is a pleiotropic cytokine produced by monocytes and macrophages [16]. IL-1Ra inhibits IL-1α- and IL-1β-mediated cellular activation. Since IL-1 is a prominent proinflammatory cytokine involved in a number of inflammatory diseases, IL-1Ra therapy has been suggested to be a potential therapeutic option in systemic inflammatory diseases [16]. IL-1Ra levels have previously been shown to increase in systemic circulation after cardiac surgery [5, 17]. IL-1Ra increased in the present study in parallel with the proinflammatory cytokines, both in cardiotomy suction blood and in the systemic circulation.

IL-4 is a multifunctional anti-inflammatory cytokine that plays an important role in the regulation of immune responses [18]. Plasma concentrations of IL-4 increase in sepsis [19]. In the present study, the plasma concentration of IL-4 was minimal at all time points, both in the systemic circulation and in cardiotomy suction blood, which confirms the results of previous studies in cardiac surgery patients [20, 21]. The results therefore illustrate the marked difference between two major inflammatory conditions (sepsis and surgery-induced inflammatory activation) at the subcellular level.

IL-10 is the most well-studied anti-inflammatory cytokine in cardiac surgery. It is a potent anti-inflammatory cytokine produced by T cells and macrophages. These cells are important in controlling inflammation initiated by proinflammatory mediators.

**DISCUSSION**

The main findings of the present study were as follows: (i) the balance of pro- and anti-inflammatory cytokines in cardiotomy suction blood is unfavourable; (ii) cell salvage of cardiotomy suction blood reduces the absolute levels of both pro- and anti-inflammatory cytokines and (iii) cell salvage improves the balance between pro- and anti-inflammatory cytokines in the systemic circulation 24 h after surgery.
(IL-6, IL-8 and TNF-α) [22, 23]. IL-10 inhibits the secretion of TNF-α, IL-1β and IL-6 and suppresses the propagation of downstream effects [22, 23]. Preoperative treatment with corticosteroids has been shown to increase IL-10 levels in blood and to reduce the proinflammatory response after CPB [4]. In the present study, IL-10 levels increased in the systemic circulation during and after surgery, which is in accordance with the results of previous studies [4, 15, 20]. However, in contrast to IL-1r, IL-6 and TNF-α levels, IL-10 levels did not increase in cardiotomy suction blood. This is a new and potentially important finding.

The lack of any increase in IL-10 in cardiotomy suction blood in combination with increased IL-6 levels also resulted in a markedly increased IL-6-to-IL-10 ratio. Recent studies have suggested that this disturbed balance between pro- and anti-inflammatory activation might be more important than the increase in individual pro-inflammatory cytokines [4-9]. Our results demonstrate further that cell-saver processing of the cardiotomy suction blood before retransfusion improves the unfavourable balance between pro- and anti-inflammatory cytokines in the systemic circulation after cardiac surgery (Table 4 and Fig. 1). Our results are supported by a study by Cholette et al. [9] on paediatric cardiac surgery patients where transfusion of washed blood products improved the IL-6-to-IL-10 balance.

Cardiotomy suction blood may be either retransfused without preceding cell-saver processing or retransfused after processing, which is recommended in the current guidelines to reduce the need for autologous red blood cell transfusions [24]. A recent study by Damgaard et al. [14] indicated that cell salvage of cardiotomy suction blood before retransfusion reduces the levels of both pro- and anti-inflammatory cytokines, but the authors did not report on the balance between pro- and anti-inflammatory responses. A third option in patients with limited intraoperative bleeding is to completely refrain from retransfusion and to discard the highly activated and fat-contaminated suction blood. There are data to suggest that this third alternative reduces the inflammatory response and preserves the haemostatic properties in the systemic circulation in comparison with retransfusion of unprocessed blood [10, 25]. However, there have been no studies directly comparing retransfusion of processed cardiotomy suction blood with a non-retransfusion policy.

The present study has important limitations. Only a limited number of pro- and anti-inflammatory cytokines were analysed, and other cytokines might have reacted differently. Furthermore, it was not possible to do a correct power analysis in the secondary, randomized part of the study. At the start of the study, no one had published any data regarding anti-inflammatory activation and the balance between pro- and anti-inflammatory activation in cardiotomy suction blood. Type-II statistical error cannot therefore be excluded, and the results from this part of the study should therefore be considered preliminary.

In summary, cardiotomy suction blood has a poor balance between pro- and anti-inflammatory activation. Cell salvage processing of cardiotomy suction blood reduces the absolute concentrations of both pro- and anti-inflammatory cytokines and improves the postoperative balance.

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