Comparison of inflammatory response following coronary revascularization with or without cardiopulmonary bypass

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BACKGROUND: It is well known that conventional coronary revascularization is associated with a pronounced systemic inflammatory response due to the application of cardiopulmonary bypass (CPB).

OBJECTIVES: To compare the effects of coronary artery bypass grafting (CABG) with (on-pump) or without (off-pump) extracorporeal circulation observing certain inflammatory response parameters.

METHODS: Twenty patients undergoing CABG with (CPB group) 10 patients or without (off-pump coronary artery bypass grafting [OPCAB] group: 10 patients) CPB were enrolled in this prospective, randomized study. Blood samples were collected three times during the operation and on postoperative days 1, 3, and 7. The plasma level of proinflammatory cytokine tumor necrosis factor (TNF)-alpha was measured by enzyme-linked immunosorbent assay method following stimulation, and the expression of adhesion molecules (CD11a, CD18) of leukocytes were determined by flow cytometry.

RESULTS: The WBC and neutrophil counts rose markedly in both groups following the operation and remained at this increased level during the observation period. There was a significant difference in WBC and neutrophil counts between the two groups of patients on postoperative day 7. A significant difference in the level of TNF-alpha was found between the two groups on postoperative day 2 (P<0.05). An intense increase was observed with CPB, which significantly exceeded the values of the OPCAB group without extracorporeal circulation in the early postoperative period. The CD11a and CD18 expressions of leukocytes decreased during the operation and on postoperative day 1; thereafter, it increased markedly. There was a significant difference in adhesion molecule expression between the two groups on postoperative day 2.

CONCLUSION: The investigation revealed that inflammatory response reactions following extracorporeal circulation could be reduced significantly using the off-pump technique.

Key Words: Adhesion molecule; Coronary artery bypass; Cytokines

Despite advances in extracorporeal perfusion, cardiopulmonary bypass (CPB) generates a whole body inflammatory reaction leading to postoperative morbidity and a prolonged hospital stay (1-3). Important features of this inflammatory response include the activation of complement proteins induced by the activator effect of a foreign surface, heparin and heparin-protein complex (3). Vasoconstriction, elevated vascular permeability, activation of neutrophil leukocytes and mast cells, aggregation of platelets and chemotaxis are facilitated by C3 and C5a proteins arising from the lysis of complement proteins (4-6). The polymorphonuclear leukocytes (PMNs) activated by CPB produce oxygen free radicals and release several proteolytic enzymes and metabolites of arachidonic acid. The activated cells are the source of the proinflammatory cytokines that enhance inflammatory reactions (3). The stimulated PMNs play an important role in reperfusion injury following CPB (6) via the expression of adhesion molecules leading to interaction between the endothelial cells, thereby extending the tissue damage. Four families of adhesion molecules regulate the interaction between endothelial cells and PMNs and the migration of leukocytes across the capillaritis selectin, integrin, immunoglobulin and cadherin families (5). Integrins have a crucial role during the phase of tight binding and the migration of PMNs. Leukocyte function-associated antigen-1 (LFA-1) belongs to the integrin family, which is made up of the complex of CD11a and CD18 molecules. LFA-1 appears to be the ligand of intercellular adhesion molecule-1 (ICAM-1), produced by the endothelial cells. The LFA-1/ICAM-1 interaction is essential for tight binding and, therefore, for the migration of PMNs into the extracellular space (5,6). The processes induced by CPB may affect myocardial function and increase the risk of complications. The increased expression of LFA-1 on leukocytes can provoke the migration of PMNs through the vessels, triggering harmful reactions in the tissues. It can also cause decreased capillary flow due to the firm adhesion of leukocytes to the capillary wall (7,8). To minimize the deleterious effects of CPB, many centers around the world perform coronary revascularization without CPB using off-pump coronary artery bypass grafting (OPCAB). The advantage of the OPCAB technique is mostly seen in the clinical data (9,10). The aim of the present prospective, randomized study was to investigate the effect of coronary bypass surgery with or without CPB on the inflammatory response parameters and on neutrophil adhesion molecule expression.
TABLE 1
Preoperative and postoperative data of patients

<table>
<thead>
<tr>
<th></th>
<th>CPB group</th>
<th>OPCAB group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.1±2.1</td>
<td>63.4±2.6</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>2/3</td>
<td>3/7</td>
</tr>
<tr>
<td>Number of grafts</td>
<td>3.0±0.18</td>
<td>3.6±0.31</td>
</tr>
<tr>
<td>Blood loss (mL)</td>
<td>859.6±200.3</td>
<td>747.6±42.0</td>
</tr>
<tr>
<td>Time in intensive care unit (h)</td>
<td>60±13.8</td>
<td>49±9.2±4.6</td>
</tr>
<tr>
<td>Time in hospital (h)</td>
<td>277±22.3</td>
<td>251±52±10</td>
</tr>
<tr>
<td>Level of troponin I during surgery (U/L)</td>
<td>0.6±0.14</td>
<td>0.7±0.08</td>
</tr>
<tr>
<td>Level of troponin I on postoperative day 1 (U/L)</td>
<td>2.6±0.62</td>
<td>4.6±0.17</td>
</tr>
<tr>
<td>Level of troponin I on postoperative day 2 (U/L)</td>
<td>2.0±0.75</td>
<td>0.2±0.08</td>
</tr>
</tbody>
</table>

CPB: Operated with cardiopulmonary bypass; OPCAB: Operated without cardiopulmonary bypass

PATIENTS AND METHODS

Cytokine release and adhesion molecule expression on white blood cells (WBCs) were assessed in 20 patients. Ten consecutive patients undergoing coronary surgery with CPB (CPB group) and 10 patients operated without extracorporeal circulation (OPCAB group) were enrolled. Clinical data of patients are presented in Table 1. The study protocol was approved by the Medical Ethics Committee of Zala Country Hospital, Hungary, and informed consent was obtained from all patients.

Exclusion criteria of the study were as follows: recent myocardial infarction (less than three months); severe respiratory re-operation; infection; immunological disease; tumor; severe or chronic renal failure; neurologic impairment; previous stroke; and coagulopathy.

Blood sampling

Blood samples were collected from the central vein at different time points during the surgery and the first postoperative week. The protocol for blood sampling in the CPB and OPCAB groups is shown in Table 2.

WBC count and troponin I level measurement

The measurement of WBC count and troponin I level was a part of the routinely used clinical investigation. The WBC count was determined in 10⁶ cells per litre (GLL), the level of troponin I in plasma was measured in units per litre (U/L).

Assessment of tumor necrosis factor-alpha

To measure the tumor necrosis factor (TNF) content, blood was collected in bottles containing 4.5 mL of sodium heparin. The blood was stimulated by 500 ng of phorbol-12-myristate-13-acetate and incubated at 37°C for 4 h. Thereafter, the samples were centrifuged at 3000 g for 10 min, following which the serum was separated into Eppendorf vials and frozen to −78°C until the measurement. The samples were measured for TNF level within three weeks according to the protocol (R&D Systems Inc., USA), and the final value was expressed in pg/mL.

Analysis of adhesion molecules

Flow cytometry was used to determine the level of adhesion molecules expressed on leukocytes. The conjugation of the blood was attenuated by sodium citrate. The samples were incubated with antibodies against CD11a and CD18 molecules (BD Biosciences, USA) for 15 min. The antibodies were stained with fluorescein isothiocyanate (FITC). The red blood cells were lysed by hypo-osmotic ammonium chloride solution (BD Biosciences, USA) for 12 min. To separate the monocytes, CD14-FITC staining was applied. Each sample was centrifuged at 500 g for 7 min. The pellet was then diluted by 1 mL of phosphate buffer solution and centrifuged once more (at 900) for 7 min. The pelleted layer was filtered by 0.9% formaldehyde solution and stored at 4°C. The samples were measured by BD FACSCalibur (BD Biosciences, USA) flow cytometer within five days. To determine the specific staining mouse anti-immunoglobulin G1/kappa was used according to the above-mentioned protocol. The results were analyzed by CellQuest software (BD Biosciences, USA), measuring the expression of adhesion molecule in arbitrary units (AU).

Correction and statistical analysis

Because marked hemodilution was present in the CPB group, a correction factor was used to determine the final value of WBC, neutrophil count and TNF according to the following formula:

\[
\text{Corrected value} = \frac{\text{Concentration} \times \text{hematocrit of control blood sample}}{\text{Hematocrit of the measured blood sample}}
\]

This formula was not used to calculate the results of flow cytometry. Data were presented as mean ± SEM. A comparison of the data between the two groups was done with unpaired Student’s t test. Comparisons between the control data were made using paired Student’s t test. Differences were considered significant at P<0.05.

RESULTS

There was no hospital mortality, pulmonary insufficiency or neurological complication in the two study groups. Table 1 shows the most important clinical data of the patients. There was a moderate difference in the initial postoperative blood loss, which was 859.6±200.3 mL in the CPB group and 474.7±64.9 mL in the OPCAB group. Troponin I measured at all time points differed significantly between the groups. On postoperative day 2, the sensitive indicator of myocardial injury was many times higher in the CPB group than in the OPCAB group (2.06±0.75 U/L versus 0.24±0.08 U/L, respectively).

WBC count and neutrophil PMN count increased rapidly after repertusion in both groups, and the highest value registered...
on postoperative day 2 in the CPB group (WBC count, 7.97±2.03×10⁹/L versus 28.20±8.57×10⁹/L). After this time point, the WBC and neutrophil count decreased slowly. The difference in WBC count between the groups proved to be significant on postoperative day 7 (P<0.05; Figure 1). The neutrophil count of the two groups differ significantly on postoperative days 3 and 7 (P<0.05; Figure 2).

The mean TNF value increased soon after surgery in the CPB group (Figure 3). It reached a maximum on postoperative day 1 when compared with baseline (360.53±306.7 pg/mL versus 205.52±92.5 pg/mL). A significant difference was also found during the first postoperative days (days 1 to 3) between the two groups of patients, with a notable increase in the CPB group.

The surface appearance of CD11a and CD18 adhesion molecules expressed by granulocytes was characteristic in both groups. The expression of CD11a and CD18 changed similarly in each population of leukocytes. The CD11a level on the surface of WBCs (Figure 4) tended to decrease in the early phase of reperfusion; thereafter, it increased. The expression of integrins was markedly higher on granulocytes from CPB patients than from OPCAB patients, especially on postoperative day 2 (48.6±5.9 AU and 71.2±9.7 AU in the OPCAB and CPB groups, respectively, P<0.05)

**DISCUSSION**

Although conventional coronary artery bypass grafting with the use of CPB is a safe and effective procedure, it is known to evoke many side effects. Pulmonary dysfunction occurs frequently, mostly caused by activation of inflammatory processes like degradation of complement proteins and aggregation of WBCs in the capillaries of the lung. Intestinal ischemia and acute abdominal complication amounted to 1%; furthermore,
neurological troubles and cognitive dysfunctions accounted for 7% to 25% of all complications due to CFB (11). These complications occur as a result of emboli made up of cholester-
ol, thrombus or air bubbles. Renal dysfunction resulting from CFB is also likely well known. Metabolic acidosis, throm-
bocytopenia, perturbation in coagulation and fibrinolysis are some times observed (11). The inflammatory reactions may play a key role in the genesis of catastrophic complications following CFB. This condition can lead to postperfusion syndrome involving fever, fluid accumulation in the intrathoracic space and decreased systemic vascular resistance (12-15). There are large numbers of studies investigating strategies to inhibit the inflam-
matory response during coronary surgery, thus attenuating the side effects of CFB. Apoptosis, corticosteroid (16), heparin-
coated circuit (17) and leukocyte depletion (18) are thought to attenuate inflammatory reactions mediated by CFB.

Recent attention has focused on the effect of the OPCAB technique working on the beating heart without extra-corporeal circulation (9,10). Several comparative studies have proved that the OPCAB surgery may reduce mortality and morbidity (9,19,20). According to these studies, the OPCAB operation is associated with notable decrease in postoperative blood loss and need for transfusion, and with a shortened time period on ventilatory support and in intensive care. Referring data in patients enrolled in our study have shown similar context. Increased WBC and PMN counts can be observed in each group however, it was more remarkable in the CFB group, reflecting the more severe inflammatory reaction. Several authors have investigated the level of proinflammatory cytokines during coronary bypass with or without CFB. Fransen et al (21) reported that the elevated proinflammatory reactions are caused by surgical trauma. They suggested that interleukin-6 is determined by the surgical invasiveness rather than because of reperfusion or myocardial injury (3). Further articles (22,23) have shown significant and long-lasting elevations of TNF just as we observed. Matana et al (22) reported that the first step in the inflammatory response to CFB is the activation of complements and PMNs, followed by the production of early proinflammatory (IL-1) cytokines and thereafter TNF. The elevated level of TNF may result in decreased contractile function and coronary flow (24). TNF provokes certain inflammatory processes, whereas further activation of PMNs and complement cascades are not associated with TNF expression (22).

We believe this study is the first to measure the expression of CD11a and CD11b molecules in patients undergoing coro-
nary surgery with or without CFB during the first postoperative week. During surgery and in the acute postoperative period (postoperative day 1), the expression of these molecules decreased in both groups. It can be explained by the adherence of PMNs expressing larger amount of integrins. In contrast, there was a marked increase in CD11a adhesion molecule expression in the CFB group, with significant differences between the two groups on postoperative day 2. The integrins allow the PMNs to pass through the capillaries by joining complementary molecules expressed by endothelial cells. The expression of endothelial cell-derived adhesion molecules can be measured by obtaining its soluble level in the plasma. Matana et al (22) performed this measurement and demonstrated the elevated level of ICAM-1 that is known to adhere to the LFA-1 (complex of CD11a and CD18). Thus, the probability of the diapedesis of PMNs is higher due to the elevated ICAM-1 level.

Based on our findings, we can suppose that the increased LFA-1 expression is a causative factor for the increased migration of PMNs through the vascular wall. Furthermore, the extreme adhesion of PMNs can lead to leukocyte aggregation (leukostasis), producing a disturbance in capillary flow.

Based on our findings and literature data, we can suppose that the extensive inflammatory response to open heart surgery results from CFB. Surgical manoeuvres, contact of blood components with an artificial surface, aortic cross-clamping and reperfusion injury are considered the main causative factors determined by the activation of neutrophils, cytokines and adhesion molecules. Preventing the activation of inflammatory response reactions with the OPCAB technique has the ability to reduce the potential mortality and morbidity of the patients with coronary artery bypass grafting by extra-corporeal circulation.

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REFERENCES