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).

OBJECTIVE: 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, 2, 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 (CD11, CD18) of leukocytes were determined by flow cytometry. Furthermore, white blood cell (WBC) and neutrophil count were carried out.

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 expression 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; Cardiopulmonary bypass; Coronary 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-protamin complex (3). Vasoconstriction, elevated vascular permeability, activation of neutrophil leukocytes and mast cells, aggregation of platelets and chemotaxis are facilitated by C3a 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 capillaries: 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 interraction 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 centres 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.

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TABLE 1
Preoperative and postoperative data of patients

	CPB group	OPCAB group
Age (years)	63.1±2.1	63.4±2.8
Sex (male/female)	2/8	3/7
Number of grafts	3.9±0.18	3.4±0.31
Blood loss (mL)	889.6±200.3	747.8±64.9
Time in intensive care unit (h)	60.0±13.9	49.3±2.4
Time in hospital (h)	277.8±23.8	251.5±12.0
Level of troponin I during surgery (U/L)	0.81±0.14	0.17±0.08
Level of troponin I on postoperative day 1 (U/L	2.63±0.62	0.41±0.17
Level of troponin I on postoperative day 2 (U/L)	2.06±0.75	0.24±0.08

CPB Operated with cardiopulmonary bypass; OPCAB Operated without cardiopulmonary bypass

TABLE 2
Protocol for blood sampling

Sample name	CPB group	OPCAB group
Control	After the induction of anesthesia	
Ischemia	During the cross-clamping of the aorta	During the performing of the last graft
Reperfusion	30 min after cessation of aorta cross-clamping	30 min after completion of the last graft
Postoperative day 1	Postoperative period	
Postoperative day 2		
Postoperative day 3		
Postoperative day 7		

CPB Operated with cardiopulmonary bypass; OPCAB Operated without cardiopulmonary bypass

PATIENTS AND METHODS

Patients

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); acute operation; re-operation; infection; immunological disease; tumour; acute or chronic renal failure; respiratory 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 10^9 cells per litre (G/L), the level of troponin I in plasma was measured in units per liter (U/L).

Assessment of tumour necrosis factor-alpha

To measure the tumour 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 –75°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 coagulation 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 lyzed 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 300 g for 7 min. The pellet was then diluted by 2 mL of phosphate buffer solution and centrifuged once more (at 900/min for 7 min). The pellet was fixed by 0.5% formaldehyde solution and stored at 4°C. The samples were measured by BD FacsCalibur (BD Biosciences, USA) flowcytometer within five days. To determine the aspecific staining mouse anti-immunoglobulin G_1-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 needed to be used to determine the final value of WBCs, neutrophil count and TNF according to the following formula:

$$\text{Corrected value} = \frac{\text{Concentration} \times \text{hematocrit of control blood sample}}{\text{Hemocrit of the measured blood sample}}$$

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 modest difference in the total postoperative blood loss, which was 889.6±200.3 mL in the CPB group and 747.77±64.9 mL in the OPCAB group. Troponin I levels measured at all time points differed significantly between the two groups. On postoperative day 2, this 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 reperfusion in both groups, and the highest value registered

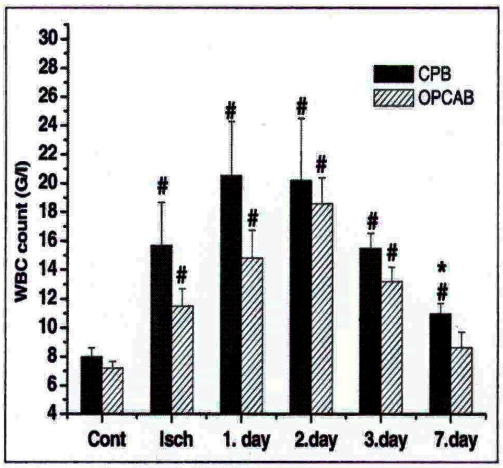

Figure 1) *White blood cell (WBC) count in patients operated with (CPB) or without (OPCAB) cardiopulmonary bypass. #P<0.05 compared with the control (Cont; preoperative) values; *P<0.05 for comparison of the two groups at the same time as when blood samples were taken. G/l 10^9 cells per litre; Isch Ischemia*

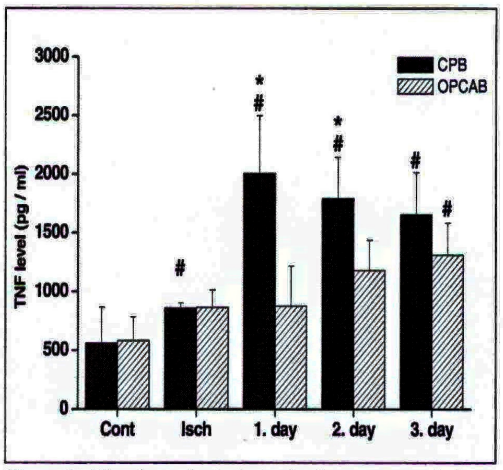

Figure 3) *The plasma level of stimulated tumour necrosis factor (TNF)-alpha in patients operated with (CPB) or without (OPCAB) cardiopulmonary bypass. #P<0.05 compared with the control (Cont; preoperative) values; *P<0.05 for comparison of the two groups at the same time as when blood samples were taken. Isch Ischemia*

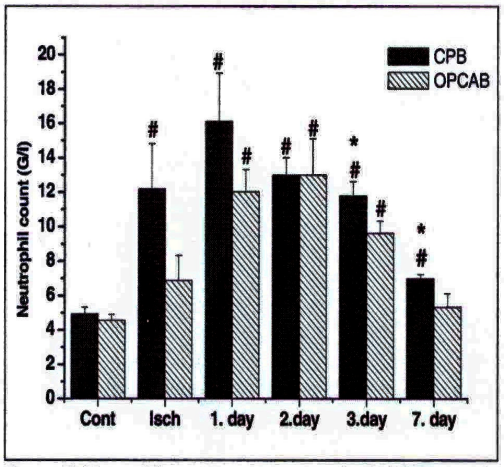

Figure 2) *Neutrophil count in patients operated with (CPB) or without (OPCAB) cardiopulmonary bypass. #P<0.05 compared with the control (Cont; preoperative) values; *P<0.05 for comparison of the two groups at the same time as when blood samples were taken. G/l 10^9 cells per litre; Isch Ischemia*

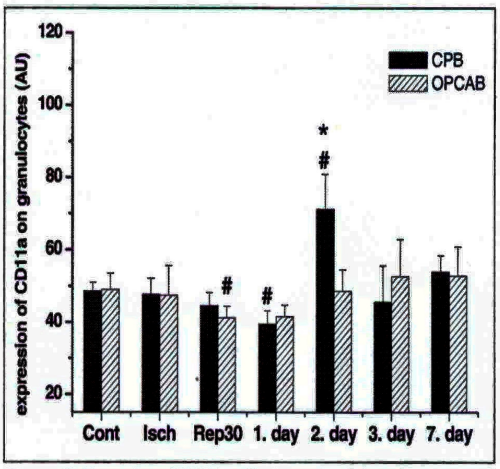

Figure 4) *Expression of CD11a adhesion molecule in patients operated with (CPB) or without (OPCAB) cardiopulmonary bypass. #P<0.05 compared with the control (Cont; preoperative) values; *P<0.05 for comparison of the two groups at the same time as when blood samples were taken. AU Arbitrary units; Isch Ischemia; Rep30 Reperfusion after 30 min*

on postoperative day 2 in the CPB group (WBC count, 7.974±2.007 G/L versus 20.20±8.57 G/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 (560.53±306.7 pg/mL versus 2005.25±492.5 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 CPB (11). These complications occur as a result of emboli made up of cholesterol, thrombus or air bubbles. Renal dysfunction resulting from CPB is also likely well known. Metabolic acidosis, thrombocytopenia, perturbation in coagulation and fibrinolysis are sometimes observed (11). The inflammatory reactions may play a key role in the genesis of catastrophic complications following CPB. This condition can lead to postperfusion syndrome involving fever, fluid accumulation in the interstitial space and decreased systemic vascular resistance (12-15). There are large numbers of studies investigating strategies to inhibit the inflammatory response during coronary surgery, thus attenuating the side effects of CPB. Aprotinin, corticosteroid (16), heparin-coated circuit (17) and leukocyte depletion (18) are thought to attenuate inflammatory reactions mediated by CPB.

Recent attention has focused on the effect of the OPCAB technique working on the beating heart without extracorporeal 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 decreases 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 CPB group, reflecting the more intensive inflammatory reaction. Several authors have investigated the level of proinflammatory cytokines during coronary bypass with or without CPB. 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 invasivity 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. Matata et al (22) reported that the first step in the inflammatory response to CPB is the activation of complements and PMNs, followed by the production of early proinflammatory (IL-8) 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 CD18 molecules in patients undergoing coronary surgery with or without CPB 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 CPB 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. Matata 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 literary data, we can suppose that the extensive inflammatory response to open heart surgery results from CPB. 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 extracorporeal circulation.

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REFERENCES

1. Wan S, LeClerc JL, Vincent JL. Inflammatory response to cardiopulmonary bypass: Mechanisms involved and possible therapeutic strategies. Chest 1997;112:676-92.
2. Wan S, LeClerc JL, Vincent JL. Cytokine response to cardiopulmonary bypass: Lessons learned from cardiac transplantation. Ann Thorac Surg 1997;116:269-76.
3. Wan S, Anthony PC. Cytokines in myocardial injury: Impact on cardiac surgical approach. Eur J Cardiothorac Surg 1999;16:107-11.
4. Jerrold H, Kenichi A. Inflammatory response to cardiopulmonary bypass. Ann Thorac Surg 2003;59:715-20.
5. Springer TA. Adhesion receptors of the immune system. Nature 1990;346:425-34.
6. Schwartz JD, Shammian P, Schwartz DS, et al. Cardiopulmonary bypass primes polymorphonuclear leukocytes. J Surg Res 1998;75:177-82.
7. Craddock PR, Hammerschmidt D, White JG, Dalmosso AP, Jacob HS. Complement (C5-a)-induced granulocyte aggregation in vitro. A possible mechanism of complement-mediated leukostasis and leukopenia. J Clin Invest 1977;60:260-4.
8. Redl H, Schlag G, Kneidinger R, Dinges HP, Davies J. Activation/adherence phenomena of leukocytes and endothelial cells in trauma and sepsis. In: Schlag G, Redl H, eds. Pathophysiology of Shock, Sepsis, and Organ Failure. Berlin: Springer-Verlag, 1993:549-63.
9. Buffolo E, de Andrade CS, Branco JN, Teles CA, Aguiar LF, Gomes WJ. Coronary artery bypass grafting without cardiopulmonary bypass. Ann Thorac Surg 1996;61:63-6.
10. Benetti FJ, Naselli G, Wood M, Geffner L. Direct myocardial revascularization without extracorporeal circulation. Experience in 700 patients. Chest 1991;100:312-6.
11. Norton JA, Bollingen RR, Lowry SF, et al, eds. Surgery. Basic Science and Clinical Evidence. Berlin: Springer Verlag, 2000:1335-8.
12. Strüber M, Cremer JT, Gohrbandt B, et al. Human cytokine responses to coronary artery bypass grafting with and without cardiopulmonary bypass. Ann Thorac Surg 1999;68:1330-5.
13. Olthof CG, Jansen PG, de Vries JP, et al. Interstitial fluid volume during cardiac surgery measured by means of a non-invasive conductivity technique. Acta Anaesthesiol Scand 1995;39:508-12.
14. Westaby S. Organ dysfunction after cardiopulmonary bypass. A systemic inflammatory reaction initiated by the extracorporeal circuit. Intensive Care Med 1987;13:89-95.
15. Zanardo G, Michielon P, Paccagnella A, et al. Acute renal failure in the patient undergoing cardiac operation. Prevalence, mortality rate,

and main risk factors. J Thorac Cardiovasc Surg 1994;107:1489-95.
16. Gott JP, Cooper WA, Schmidt FE Jr, et al. Modifying risk for extracorporeal circulation: Trial of four antiinflammatory strategies. Ann Thorac Surg 1998;66:747-53.
17. Moen O, Hogasen K, Fosse E, et al. Attenuation of changes in leukocyte surface markers and complement activation with heparin-coated cardiopulmonary bypass. Ann Thorac Surg 1997;63:105-11.
18. Gu YJ, de Vries AJ, Boonstra PW, van Oeveren W. Leukocyte depletion results in improved lung function and reduced inflammatory response after cardiac surgery. J Thorac Cardiovasc Surg 1996;112:494-500.
19. Bucerius J, Gummert JF, Walther T, et al. Predictors of prolonged ICU stay after on-pump versus off-pump coronary artery bypass grafting. Intensive Care Med 2004;30:88-95.
20. Al-Ruzzeh S, Ambler G, Asimakopoulos G, et al; United Kingdom Multi-Center Comparative Analysis of Early Clinical Outcome. Off-pump coronary artery bypass (OPCAB) surgery reduces risk-stratified morbidity and mortality: A United Kingdom multi-center comparative analysis of early clinical outcome. Circulation 2003;108(Suppl 1):II1-8.
21. Fransen E, Maessen J, Dentener M, Senden N, Geskes G, Buurman W. Systemic inflammation present in patients undergoing CABG without extracorporeal circulation. Chest 1998;113:1290-5.
22. Matata BM, Sosnowski AW, Galinanes M. Off-pump bypass graft operation significantly reduces oxidative stress and inflammation. Ann Thorac Surg 2000;69:785-91.
23. Schulze C, Conrad N, Schutz A, et al. Reduced expression of systemic proinflammatory cytokines after off-pump versus conventional coronary artery bypass grafting. Thorac Cardiovasc Surg 2000;48:364-9.
24. Meldrum DR, Cleveland JC Jr, Cain BS, Meng X, Harken AH. Increased myocardial tumor necrosis factor-alpha in crystalloid-perfused model of cardiac ischemia-reperfusion injury. Ann Thorac Surg 1998;65:439-43.