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The effect of neutrophil depletion from blood cardioplegia on myocardial ischemia/reperfusion injury

Nötrofillerden arınmış kan kardiyoplejisinin miyokardiyal iskemi/reperfüzyon hasarına etkisi

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Background: We investigated whether depleting neutrophils from blood cardioplegia had a protective role against ischemia/reperfusion injury.

Methods: The study included 16 male patients who underwent coronary artery bypass grafting (CABG) and had good left ventricular functions. The patients were randomly divided into two groups equal in number, depending on the administration of antegrade/retrograde cold blood cardioplegia with or without a leukocyte filter. To determine tissue myeloperoxidase (MPO) activity, an indicator of neutrophil accumulation, biopsies were obtained from the interventricular septum after institution of cardiopulmonary bypass (CPB), before placing the aortic cross-clamp, and one hour after its removal. Cardiac indices were calculated before the institution of CPB and four hours after the removal of the aortic cross-clamp. Creatine kinase-MB (CK-MB) activity was measured every six hours on the day of the operation.

Results: With the use of filtering, the mean leukocyte count in cardioplegia was significantly reduced ($p<0.001$). The two groups did not differ significantly with respect to cardiac indices, CK-MB levels, mechanical support, development of severe ventricular arrhythmias, and the use of inotropic agents ($p>0.05$). Tissue MPO activities before aortic cross-clamping were 0.13±0.04 U/100 mg tissue and 0.11±0.04 U/100 mg tissue with and without leukocyte filtering, respectively. One hour after aortic cross-clamp removal, MPO activity showed a significant increase in both groups. Leukocyte filtering was associated with a smaller increase in MPO activity, but this did not reach significance ($p>0.05$).

Conclusion: Depletion of neutrophils from blood cardioplegia in patients undergoing elective CABG with good left ventricular functions yielded no additional benefits in terms of hemodynamic or biochemical parameters.

Key words: Cardioplegic solutions; cardiopulmonary bypass; filtration; leukocytes; myocardial reperfusion injury.

Amaç: Nötrofillerden arınmış kan kardiyoplejisinin iskemi/reperfüzyon hasarına karşı koruyucu rolü olup olmadığı araştırıldı.

Çalışma planı: Çalışmaya, koroner arter bypass greftleme (KABG) uygulanan ve sol ventrikül fonksiyonları iyi olan 16 erkek hasta alındı. Hastalar rastgele olarak, sayıca eşit iki gruba ayrıldı. Bir gruba antegrad/retrograd soğuk kan kardiyoplejisi lökosit filtresi takılarak, diğer gruba ise filtre takılmadan uygulandı. Dokuda nötrofil birikiminin belirteci olan doku miyeloperoksidaz (MPO) aktivitesinin belirlenmesi için, kardiyopulmoner bypass başlangıcında, kros klemp konmadan hemen önce ve kros klempin çıkarılmasından bir saat sonra interventriküler septumdan biyopsi örnekleri alındı. Kardiyopulmoner bypass öncesinde ve kros klempin çıkarılmasından dört saat sonra hastaların kardiyak indeksleri hesaplandı. Kreatin kinaz-MB (CK-MB) aktivitesi ameliyat gününde altı saatte bir ölçüldü.

Bulgular: Filtreleme yapılan grupta kardiyopleji sıvısındaki ortalama lökosit sayısı anlamlı derecede düşük bulundu ($p<0.001$). İki grup arasında kardiyak indeksler, CK-MB düzeyleri, mekanik destek, ciddi ventrikül aritmisi gelişimi ve inotropik ajan ihtiyacı açısından anlamlı farklılık bulunmadı ($p>0.05$). Kros klemp öncesinde, lökosit filtresi uygulanan ve uygulanmayan gruplarda doku MPO aktivitesi sırasıyla 0.13±0.04 U/100 mg doku ve 0.11±0.04 U/100 mg doku bulundu. Kros klempin çıkarılmasından bir saat sonra, MPO aktivitesi her iki grupta da anlamlı artış gösterdi. Lökosit filtresi uygulanan grupta MPO aktivitesindeki artış daha düşük olmasına karşın bu farklılık anlamlı değildi ($p>0.05$).

Sonuç: Sol ventrikül fonksiyonları iyi olan ve elektif KABG ameliyatı yapılacak hastalarda, kan kardiyoplejisinden nötrofilleri arındırmak, hemodinamik ve biyokimyasal parametreler açısından ek yarar sağlamamaktadır.

Anahtar sözcükler: Kardiyoplejik solüsyon; kardiyopulmoner bypass; filtreleme; lökosit; miyokardiyal reperfüzyon hasarı.

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It is well-known that endothelial cells play a key role in ischemia/reperfusion injury. Inflammatory response caused by hypoxia leads to some changes in the phenotype of endothelial cells, known as endothelial cell activation which accounts for the basis of ischemia/reperfusion injury. The initial hypoxia-induced change in the endothelium is the accumulation of neutrophils in this region upon the appearance of adhesion molecules such as P, E, and L selectins on the endothelial cell surface. Neutrophils are attached to endothelial cells more strongly in the presence of adhesion molecules such as intercellular adhesion molecule (ICAM) from immunoglobulin gene family and vascular cell adhesion molecule (VCAM) that forms later on. These tightly attached neutrophils reach reperfused tissues through endothelial combinations by transendothelial passage.[1] They not only directly damage the tissue by producing free oxygen radicals and proteolytic enzymes, but also create a plug at the capillary level that spoils perfusion. The primary appearance of this final picture is known as no-reflow phenomenon characterized by a blockage of the capillary bed by neutrophils sticking together to the endothelium.[1,2]

Despite the presence of endothelial activation throughout the ischemic period, neutrophil-induced injury does not occur until reperfusion occurs. The purpose of our study was to determine whether depletion of neutrophils from blood cardioplegic solutions provided any protection against ischemia/reperfusion injury.

PATIENTS AND METHODS

Sixteen male patients were included in our study. All the patients had good left ventricular functions and underwent coronary artery bypass grafting (CABG) by the same team. All the patients were informed about the study and their consent was obtained. Exclusion criteria included a history of diabetes, reoperation, emergency operation, and the presence of left ventricular ejection fraction (LVEF) <30%.

Anesthesia. Premedication comprised intramuscular midazolam 3 mg and scopolamine 0.5 mg. Induction was attained by fentanyl citrate 15 µg/kg and pancuronium bromide 0.1 mg/kg. Maintenance was with fentanyl citrate 7 µg/kg/hr and propofol 2 mg/kg/hr with hourly administration of intravenous pancuronium bromide 2 mg and an inhaler anesthetic (sevoflurane). Phenylephrine and nitroglycerin were used intraoperatively when needed so as to keep arterial blood pressure within acceptable limits.

Surgical technique. A standard midsternal incision was used in all the patients. Left internal thoracic artery flap (LITA), saphenous vein grafts, and in some selected patients, left radial artery grafts were prepared. A Y-shaped cannula was placed above the ascending aorta for antegrade cardioplegia and venting. The coronary sinus was cannulated for retrograde cardioplegia. A centrifugal pump and membrane oxygenator were used in all the patients. Cardiopulmonary bypass (CPB) was instituted and systemic body temperature was reduced to 30 °C. The patients were randomly divided into two groups equal in number. Group I received antegrade/retrograde cold blood cardioplegia through a leukocyte filter (Bio R 01 Max, Fresenius AG, Oberursel, Germany) placed at the inlet of the cardioplegia infusion set. Group II received antegrade/retrograde cold blood cardioplegia without the use of a leukocyte filter.

The cardioplegia used was prepared by adding 20 mEq K^+,16 mEq HCO_3^-, 7.164 mg citrate, 16 mmol Mg^{++} and 1 gr glucose to 1 liter of arterial blood taken from the patients after the cannulation of the ascending aorta. As soon as the cross-clamp was placed, 10 ml/kg cardioplegia was initially administered, 2/3 from the antegrade path, and the remaining 1/3 from the retrograde path. The solution was re-administered retrogradely with a pressure not exceeding 40 mmHg with 20 minute intervals. Following each distal anastomosis, cold blood cardioplegia was applied from the graft under standard pressure and flow. In group I, the effectiveness of filtration was checked by taking blood samples on both sides of the filter towards the end of antegrade cardioplegia. Following the completion of distal anastomoses, proximal anastomoses were carried out with the single cross-clamp technique. Cardiopulmonary bypass was terminated when rectal temperature was 36.5 °C. Protamine was administered for heparin by slow infusion in a regimen of 1:1.

Clinical parameters. Cardiac indices (CI) of the patients were calculated by the Fick method before the institution of CPB and four hours after the removal of the aortic cross-clamp. Postoperative arrhythmias, mechanical and pharmacological inotropic needs were recorded.

Blood and biopsy samples. Peak values of creatine kinase-MB (CK-MB) were measured in every patient every six hours on the day of the operation.

Biopsies were obtained from the interventricular septum with a 14-gauge 20-mm tru-cut automatic biopsy needle (Gallini Medical, Mantova, Italy) after institution of CPB, before placing cross-clamp, and one hour after the removal of the cross-clamp. The samples were kept in a sucrose solution of 0.6 mol/l at -85 °C until evaluation. Tissue myeloperoxidase (MPO) activity, an indicator of neutrophil accumulation in the tissue,[1] was determined in the biopsy samples in the laboratories of the Pharmacology Division of the Pharmacy Faculty of Marmara University.

Table 1. Patient characteristics and operative data

	Group I (n=8)		Group II (n=8)	
	n	Mean±SD	n	Mean±SD
Age (years)		62±12		60±9
Preoperative left ventricular ejection fraction (%)		52±6		53±7
Bypass grafts		2.6±0.5		2.7±0.4
Use of left internal thoracic artery	8		8	
Use of saphenous vein	12		12	
Use of radial artery	1		2	
Cross-clamp time (min)		70±11		68±10
Cardiopulmonary bypass time (min)		97±15		101±18
Dose of cardioplegia (ml)		1,325±88		1,275±128

Detection of tissue MPO activity: Tissue MPO activities were determined by the method defined by Bradley et al. and modified by Mullane et al.[3] Biopsy samples were homogenized in 50 mmol/l of potassium phosphate buffer (pH 6) containing 0.5% HTAB (hexadecyl trimethylammonium bromide) by an Ultra-Turrax T-25 homogenizator (Janke & Kunkel IKA-Labortechnic, Staufen, Germany) for 60 seconds at a speed of 9500 rpm. One milliliter of 0.5% homogenate was transferred into 1.5 ml Eppendorf tubes. After three cycles of freezing (-85 °C) and thawing, the homogenate was centrifuged at 12500 x g at 4 °C (Heraeus, Biofuge-Pico, Hanau, Germany). Supernatants were put into reaction in 50 mmol/l potassium phosphate buffer pH 6 containing 0.167 mg/ml o-dianisidine dihydrochloride and 0.05 M H_2O_2.

Measurements were made at 460 nm by a spectrophotometer (Shimadzu UV-1208, UV-VIS, Kyoto, Japan). One unit of MPO activity was defined as the amount of enzyme hydrolyzing 1 mmol of peroxide per minute at 37 °C.

Statistical analysis. All data were indicated as mean±standard deviation. The chi-square test was used to compare nonparametric variables (saphenous, LITA, radial artery use, severe ventricular arrhythmia and inotropic requirement when separating from CPB) of the two groups. Parametric variables (age, LVEF, number of grafts used for bypass, cross-clamp time, CPB time, amount of cardioplegia, pre-CPB CI, 4th hour CI after cross-clamp, CK-MB activity) between groups were analyzed using the Mann-Whitney U-test. In group I, leukocyte counts in the cardioplegia solution on both sides of the filter were evaluated by paired samples t-test. In group I and II, MPO activities determined before and after an hour of aortic cross-clamp removal were compared using analysis of variance (ANOVA) and Tukey's test. Statistical calculations were made with the GraftPod Prism program. P values of less than 0.05 were considered statistically significant.

RESULTS

There were no significant differences between the two groups with respect to age, LVEF, number of bypasses, grafts used, cross-clamp time, CPB time, and the amount of cardioplegia administered (Table 1). No technical difficulty was encountered during biopsies or the use of leukocyte filters. In group I, the mean postfiltration leukocyte count in cardioplegia was significantly reduced (prefiltration: 6,763±346 cell/mm^3, postfiltration: 400±12 cell/mm^3, $p<0.001$).

Clinical parameters. The average CI values in group I and II before CPB were 4.5±0.8 L/min/m^2 and 4.3±0.7 L/min/m^2, respectively. They decreased to 3.2±0.7 L/min/m^2 and 2.7±0.3 L/min/m^2, respectively, four hours after the removal of the aortic cross-clamp. Pre- and postprocedural CIs did not differ significantly between the two groups ($p>0.05$).

None of the patients required mechanical support. Only one patient in each group required inotropic support (dobutamine, 8 and 6 µg/kg/min) during weaning from CPB. At the end of 24 hours, both patients needed no inotropic support. In group I, no severe ventricular arrhythmias were seen postoperatively. In group II, one patient received lidocaine perfusion due to ventricular extrasystolic arrhythmias that developed on the operation day. No significant differences were found between the two groups in terms of development of severe ventricular arrhythmias and the use of inotropic agents ($p>0.05$).

Blood and biopsy samples. The mean peak CK-MB level recorded in the first 24 postoperative hours was 47±15 IU/l in group I and 58±11 IU/l in group II. Although the increase in CK-MB was less in group I, there was no significant difference between the two groups.

Tissue MPO activity before aortic cross-clamping was 0.13±0.04 U/100 mg tissue in group I and 0.11±0.04 U/100 mg tissue in group II. One hour after

aortic cross-clamp removal, MPO activity showed a significant increase in both groups, being 0.23±0.06 U/100 mg tissue and 0.28±0.04 U/100 mg tissue, respectively. The increase in MPO activity was less in group I, but this did not reach significance.

DISCUSSION

The protective effect of leukocyte filtration during reperfusion, though short-lasting, is well known. P-selectin-mediated early adhesion is a critical stage that defines the severity of reperfusion injury. Neutrophils held on endothelial cell surfaces by P-selectins further increase secretion of P-selectin by producing free oxygen radicals, resulting in a greater number of neutrophils adhering to the endothelium. At this stage, removal of neutrophils from the reperfusate is associated with decreased P-selectin production with an ultimate alleviating effect on ischemia/reperfusion injury, even if neutrophils may reenter the media from the circulation afterwards.[4] Westlin and Mullane[5] demonstrated this beneficial role of neutrophil depletion in an experimental model. In the light of this information, we tested the hypothesis that separation of neutrophils from blood cardioplegia might mitigate reperfusion injury. However, comparison of the two groups showed that filtering neutrophils did not provide any additional hemodynamic or biochemical benefits.

In a previous study, Roth et al.[6] found that the use of blood cardioplegia with filtered neutrophils in patients with severe left ventricular dysfunction, increased LVEF significantly at 60 minutes after CPB. In another study by Sawa et al.[7] leukocyte filtering was used only during infusion of terminal blood cardioplegia. Although leukocyte filtration provided no additional benefit in patients undergoing elective CABG, it significantly reduced the need for postoperative inotropic use and decreased CK-MB activity in patients undergoing emergency CABG due to cardiogenic shock. Similarly, Sawa et al.[8] reported that leukocyte depletion was associated with a decrease in reperfusion injury in patients with left ventricular hypertrophy undergoing aortic valve replacement. In a recent study, Hayashi et al.[9] applied neutrophil filtration during the application of terminal blood cardioplegia in patients undergoing aortic valve replacement. They reported that neutrophil filtration showed no beneficial effect in patients undergoing aortic cross-clamping for less than 120 minutes, but at extended cross-clamp times, it reduced neutrophil-mediated myocardial injury.

The results of the above-mentioned studies suggest that separating neutrophils from blood cardioplegia can reduce reperfusion injury only in cases with hypertrophic ventricles, in patients undergoing emergent CABG operations, and at extended aortic cross-clamp times. This effect is easy to explain in emergent cases with extended cross-clamp times. Penetration of neutrophils into the tissue requires some changes related with hypoxia in the endothelium. In elective cases, such changes seem to appear upon ischemia caused by aortic cross-clamping and increased endothelial cell activation depends on the duration of ischemia. In emergent cases, however, this activation is already present before the operation due to critical ischemia.

It should be noted that a major proportion of cardioplegia is administered in the initial dose at a time endothelial activation related to aortic cross-clamping has yet to start. For this reason, blood cardioplegia depleted from neutrophils may be reducing ischemia/reperfusion injury in emergent CABG operations and extended aortic cross-clamping as opposed to elective CABG operations. Its protective effect in patients with poor cardiac conditions or hypertrophic ventricles may arise from the sensitivity of these patients to ischemia/reperfusion injury.

In most of the studies, neutrophil filtration is performed only during the administration of terminal blood cardioplegia. Generally, we use terminal blood cardioplegia in cases with extended aortic cross-clamp times. None of the patients received terminal blood cardioplegia in our study. It is reasonable to apply controlled reperfusion together with neutrophil filtration immediately before the aortic cross-clamp is removed and during culmination of endothelial cell activation. Nevertheless, this strategy alone has been demonstrated not to reduce reperfusion injury by Hayashi et al.[9] In elective cases and in patients with aortic cross-clamp times shorter than 120 minutes, neutrophil filtration was of no use even when it was applied during the administration of terminal cardioplegia.[9]

In conclusion, depletion of neutrophils from blood cardioplegia provides no additional hemodynamic benefit in elective patients with normal ventricle functions. However, taking into account its ease of administration, it may be kept in mind particularly in emergent CABG operations or in cases with extended aortic cross-clamping.

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