INFLUENCE OF DURAFLO II HEPARIN-TREATED EXTRACORPOREAL CIRCUITS ON THE SYSTEMIC INFLAMMATORY RESPONSE IN PATIENTS HAVING CORONARY BYPASS

Cardiopulmonary bypass generates a systemic inflammatory response, including the activation of leukocytes, contributing to postoperative morbidity. To evaluate whether the use of heparin-treated extracorporeal circuits could reduce the inflammatory reaction in patients undergoing cardiopulmonary bypass, we conducted a prospective clinical study on 14 patients having coronary artery bypass in whom perfusion was done randomly with either Duraflo II heparin-treated circuits or with nontreated circuits. In both groups systemic heparinization was performed before cardiopulmonary bypass. The use of heparin-treated circuits resulted in a reduction of systemic inflammatory activation during cardiopulmonary bypass. This was reflected by lower plasma levels of soluble tumor necrosis factor receptors ($p < 0.05$) and of interleukin-6 and interleukin-8 ($p < 0.05$), manifest after release of the aortic crossclamp. Furthermore, 6 and 12 hours after aortic crossclamp release significantly lower levels of the soluble E-selectin ($p < 0.05$) were observed in the Duraflo II group. In patients in whom noncoated circuits were used, a significant decrease in circulating soluble intercellular adhesion molecule 1 ($p < 0.05$) was found early during bypass. All these observations suggest that the use of a heparin-treated extracorporeal circuit reduces the systemic inflammatory activation and may alter the leukocyte-endothelium interaction. (J THORAC CARDIOVASC SURG 1995;110:1633-41)

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During cardiopulmonary bypass (CPB), leukocyte contact with synthetic surfaces initiates a host defense reaction with the characteristics of a systemic inflammatory response syndrome. Leukocyte activation during CPB includes release of proteolytic enzymes and changes in leukocyte adhesiveness and leukocyte sequestration in tissue beds, especially in the lungs, with the potential danger of microvascular plugging and capillary leakage. Besides contact activation, ischemia-reperfusion injury, the reinfusion of shed blood, and hemodilution may

contribute to the increased leukocyte adherence to vascular endothelium. The adhesion of leukocytes to endothelium creates a microenvironment in which damaging products of activated leukocytes are released in close contact with vascular endothelium, which results in further endothelial activation or even endothelial injury.¹⁻⁴

Although the large size of leukocytes and their relative lack of deformability make these cells easy to be trapped physically within the endothelial lining,⁵⁻⁷ the interaction between leukocytes and endothelium is largely mediated by biochemical processes.⁸⁻¹⁰ The adhesive interactions between leukocytes and endothelial cells are mediated in large part by specific leukocyte and endothelial adhesion molecules.¹¹⁻¹⁵ It has been shown that the adhesion molecules such as soluble intercellular adhesion molecule 1 (sICAM-1) and E-selectin are inducible on the endothelial surface.¹⁶⁻¹⁹ The proinflammatory cytokines tumor necrosis factor (TNF), interleukin-6 (IL-6), and interleukin-8 (IL-8) are known to stimulate endothelial cells to increase the

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expression of these molecules and thus facilitate leukocyte adherence.²⁰⁻²² Moreover, IL-8 is a major neutrophil chemotactic and activating factor produced by various types of human cells.

Modification of the material surface of the heart-lung machine has been recognized as a technique to change the interaction with blood. Originally, this method was designed to allow application of thromboresistant characteristics to the materials.²³⁻²⁶ Later, it was found that heparin coating of the material surface reduced complement activation and might thus bear the potential to change leukocyte activation^{27, 28} and subsequently the leukocyte-endothelium interaction. Therefore, the purpose of this study was to evaluate whether the use of heparin-treated (Duraflo II surface, Bentley Laboratories Division, Baxter Healthcare Corp., Irvine, Calif.) extracorporeal circuits could reduce the leukocyte-mediated inflammatory response in patients undergoing CPB.

Patients and methods

Patients. Fourteen adult patients subsequently undergoing elective coronary artery bypass grafting were enrolled in this study. The patients were randomly divided into two groups with (A, $n = 7$) or without (B, $n = 7$) the use of a heparin-treated extracorporeal circuit. Exclusion criteria were the following: previous cardiac surgery, congestive cardiac failure, neurologic disorders (e.g., cerebrovascular accident), severe pulmonary disorders (e.g., chronic obstructive pulmonary disease, emphysema), diabetes, renal diseases (e.g., renal failure), liver diseases, and preoperative coagulopathies. Informed consent was obtained from each patient the day before the operation. The study was approved by the local ethical and research council.

Anesthesia and monitoring. Standard anesthetics (lorazepam, fentanyl citrate, sufentanil citrate, alfentanil hydrochloride, midazolam hydrochloride, pancuronium bromide) and monitoring techniques (electrocardiogram, central venous-pulmonary arterial pressure monitoring, urinary output, rectal and skin temperature monitoring) were used in both groups. Cefuroxime was used for antibiotic treatment, and the first dose was administered before sternotomy.

Before connection of the extracorporeal circuit for CPB, heparin (300 IU/kg, Heparin Leo, Leo Pharmaceutical Products BV, Weesp, The Netherlands) was administered to achieve an activated coagulation time (ACT) greater than 480 seconds (Hemochron 400, International Technidyne Corp., Edison, N.J.).

CPB. The main components in the extracorporeal circuit consisted of a hollow-fiber membrane oxygenator (Univox, Baxter Bentley), a venous reservoir (BMR-1900, Baxter Bentley), a cardiomy reservoir (in group A: BCR-3500 Gold, Baxter Bentley; in group B: William Harvey H4700; C. R. Bard Inc., Tewksbury, Mass.), and

an arterial line filter (in group A: AF 1040 Gold, Baxter Bentley; in group B: Sartorius; Sartorius AG, Göttingen, Germany). In group A, all components exposed to blood were pretreated with heparin-bonded coating (Duraflo II). The standard priming of the extracorporeal circuit was 1300 ml Haemaccel 3.5% solution (Behringwerke AG, Marburg, Germany), 200 ml mannitol 20%, 100 ml human albumin 20%, 50 ml NaHCO₃ 8.4%, 20 ml KCl 7.45% (B. Braun Medica BV, Uden, The Netherlands), and 6500 IU Heparin Leo. After institution of CPB at a flow rate of 2.4 L/min per square meter and after a blood temperature below 28° C (25° to 28° C) had been reached, the heart was typically cooled with 4° C saline solution 0.9% until it fibrillated. The aorta was then crossclamped, and a single dose of approximately 800 ml (600 to 1000 ml) of St. Thomas' Hospital No. 1 cardioplegic solution at 4° C was infused into the aortic root during a 4-minute period (3 to 5 minutes) to provide myocardial preservation. Topic cooling was maintained during the infusion of the cardioplegic solution.

Target flow rates of 2.4 L/min per square meter were maintained at normothermia and correspondingly lower rates of 2.0 L/min per square meter (1.8 to 2.2 L/min per square meter) were maintained at moderate hypothermia (28° C), depending on the venous oxygen saturation and the arterial pressure. Pulsatile perfusion was used throughout the period of aortic crossclamping. During the conduct of CPB, attention was given to restrict and, if possible, to avoid the use of cardiomy suction. Cardiomy suction and aortic root venting comprised in all cases less than 2% of the calculated flow (<90 ml/min, intermittent). A volume pressure control unit was used, with negative pressures less than 60 mm Hg. In all patients the left anterior descending coronary artery was revascularized with the left internal mammary artery. Papaverine was locally applied to the mammary artery after dissection to prevent spasm. After completion of all the distal anastomoses, the aortic crossclamp was removed and the proximal anastomosis was performed with a partial occlusion clamp after spontaneous or electrical defibrillation, while rewarming of the patient to 37° C continued. Additional heparin was administered during CPB if the ACT was lower than 400 seconds. After CPB, heparin was reversed by a 3 mg/kg dose of protamine chloride (Hoffman-La Roche BV, Mijdrecht, The Netherlands). Reversal of the heparin effect was determined by heparin/protamine titration (heparin assay cartridges, Medtronic HemoTec, Inc., Englewood, Colo.) on the Hepcon System-Four (Medtronic HemoTec). All pump blood was returned to the patient through the aortic cannula or intravenously via infusion bags without hemoconcentration.

Plasma samples. Blood samples were taken after induction of anesthesia, before aortic crossclamping, after release of the aortic crossclamp (i.e., start reperfusion), and 1/2, 1 1/2, 3, 6, 12, and 24 hours after release of the aortic crossclamp. Blood was collected in evacuated blood collection tubes containing ethylenediaminetetraacetic acid. Plasma was separated from blood cells by centrifugation at 1000 g for 5 minutes and stored at -70° C until analysis.

Measurement of inflammatory mediators. Inflammatory mediators were measured with sandwich ELISAs,* which have been described elsewhere.²⁹⁻³² In short, immunoassay plates (Nunc-Immuno Plate Maxi-sorp, Roskilde, Denmark) were coated with monoclonal antibodies MR1-1, MR2-2, HM2, and ENA1 for measurement of soluble TNF receptors sTNF-R55, sTNF-R75, sICAM-1, and sE-selectin, respectively. After being washed, the plates were incubated with plasma samples followed by incubation with specific biotin-labeled polyclonal rabbit anti-sTNF-R immunoglobulin G (IgG), biotin-labeled monoclonal antibody HM1, and monoclonal antibody ENA2, for sICAM-1 and sE-selectin detection, respectively. After the plates were washed, peroxidase-labeled streptavidin (Dako, Glostrup, Denmark) was added, and 3,3',5,5'-tetramethylbenzidine (Kirkegaard & Perry Lab., Gaithersburg, Md.) was used as a substrate. Photospectrometry (450 nm) was performed with a micro-ELISA autoreader. Standard curves were constructed using dilutions of human sTNF-R55, sTNF-R75, sICAM-1, and sE-selectin with known concentrations. The lower detection limits of the assays were 100 pg/ml for TNF-Rs, 400 pg/ml for sICAM-1, and 1 ng/ml for sE-selectin. Similarly, ELISAs were used to determine plasma IL-6, IL-8, and TNF- α levels. For this purpose, plates were coated with monoclonal antibodies 5E1, HM5, and 61E71, respectively.

On incubation with plasma samples, the IL-6 and TNF- α assays were incubated with polyclonal rabbit antihuman IL-6 IgG or polyclonal rabbit antihuman TNF- α IgG followed by peroxidase-conjugated goat antirabbit IgG (Jackson ImmunoResearch, Westgrove, Pa.). For the detection of IL-8, biotin-labeled polyclonal rabbit antihuman IL-8 followed by streptavidin-peroxydase conjugate was used. In these assays tetramethylbenzidine was used as a substrate. Human recombinant IL-6, human recombinant IL-8, and human TNF- α were used for standard titration curves. The lower detection level was 10 pg/ml for IL-6, 100 pg/ml for IL-8, and 20 pg/ml for TNF- α .

Statistics. The results were expressed as the mean $\pm$ standard error. The Wilcoxon rank sum test was used for statistical analysis of differences between both experimental groups. The Wilcoxon matched-pairs signed-rank test was used for analysis of differences between control values and different time points within one group. A *p* value of less than 0.05 was considered to indicate a statistically significant difference between measured values.

Results

No significant difference existed between the two groups as related to sex, age, body weight, height, CPB time, and aortic crossclamp time (Table I).

Cytokines. Changes in IL-6 plasma concentrations with time in groups A and B are shown in Fig. 1. In the patients who underwent CPB without heparin-coated extracorporeal circuits (group B), a

Table I. Clinical characteristics of the patients groups

	Duraflo II (group A)	Control (group B)
No.	7	7
Sex (M/F)	6/1	6/1
Age (yr)	58 $\pm$ 12	61 $\pm$ 11
Height (cm)	176 $\pm$ 9	173 $\pm$ 14
Weight (kg)	86 $\pm$ 14	78 $\pm$ 8
Bypass time (min)	82 $\pm$ 24	82 $\pm$ 20
Aortic crossclamp time (min)	51 $\pm$ 18	53 $\pm$ 20

No significant difference existed between the two groups. The values are expressed in mean $\pm$ standard deviation.

rise in IL-6 concentration from the preoperative level of less than 10 pg/ml was detected from the time of the start of reperfusion. After the peak value of 1.60 ± 1.31 ng/ml, which occurred 1½ hours after the start of reperfusion, concentrations declined to preoperative values 24 hours after the release of the aortic crossclamp. In contrast, a modest increase in plasma IL-6 levels was observed in patients treated with the heparin-coated extracorporeal circuits (group A). IL-6 levels rose to 0.36 ± 0.06 ng/ml 12 hours after reperfusion and then gradually declined to preoperative levels.

As soon as CPB was started, circulating IL-8 levels rose in patients of group B. A profound increase, however, was not found until the start of reperfusion. A peak level of 0.65 ± 0.55 (ng/ml) was reached at 1½ hours of reperfusion. Then plasma levels rapidly declined. Notably, in three of seven patients IL-8 levels were still elevated at 24 hours. In patients treated with the heparin-coated circuit (group A), a completely different pattern was observed. During the operation and early during reperfusion, no change in IL-8 levels occurred. At 6 hours after reperfusion, a modest increase in IL-8 of 0.21 ± 0.18 ng/ml was found. At 24 hours after the start of reperfusion no significant differences were observed between the control and the Duraflo II group (Fig. 2).

TNF, sTNF-R55, and sTNF-R75. In the present study no increase in TNF- α levels could be demonstrated (not shown). In only one patient, belonging to group B, was the detection level of our assay reached. The peak level was 74 pg/ml and this level was found ½ hour after reperfusion (data not shown). Interestingly, in this patient peak levels of soluble endothelial cell adhesion molecule, sTNF-R55, sTNF-R75, and IL-8, but not sICAM, were higher than the peak levels of these cytokines in any other patient.

*ELISA = Enzyme-linked immunosorbent assay.

Fig. 1. Circulating plasma levels of IL-6, mean ($\pm$ standard error), in patients who underwent CPB with coated (A, hatched bars) and noncoated (B, cross-hatched bars) circuits. Statistically significant differences ($p < 0.05$) between the groups are indicated with an asterisk. X-clamp, Crossclamp.

Preoperative levels of sTNF-R were not significantly different between the two groups. CPB caused increases in plasma levels of both sTNF-R. However, kinetics of sTNF-R levels were different between the two groups. In group B (extracorporeal circuit without heparin coating), sTNF-R levels started to increase as early as during CPB. sTNF-R remained elevated from 1/2 to 6 hours after release of the aortic clamp and then slowly declined again. This pattern was similar for both sTNF-Rs. Enhancement of sTNF-R levels in plasma of patients with a heparin-coated circuit was significantly delayed: no increase was found until 3 hours after release of the aortic clamp. From 6 to 24 hours onward, TNF-R levels were in the same range in both groups (Fig. 3, A and B).

sICAM and sE-selectin. Wide variations in preoperative plasma sICAM-1 levels (ranging from 80 to 442 ng/ml) were found in patients who underwent CPB without heparin-coated extracorporeal circuits (group B). After aortic crossclamping, sICAM-1 levels fell immediately to 70 ± 12 ng/ml (group B). In group A only a modest decrease was demonstrated (from 73 ± 9 to 54 ± 7 ng/ml). Twenty-four hours after CPB, the plasma level of sICAM-1 increased in both groups (group A: 107 ± 14 ng/ml; group B: 103 ± 17 ng/ml).

During the operation and subsequently during early reperfusion, circulating E-selectin levels showed a tendency to decrease without reaching a level of significance (Fig. 4). Later during reperfusion a gradual though profound increase was observed in both groups, with peak values between 12 and 24 hours of reperfusion. In patients with a noncoated circuit these peak levels were significantly higher ($p < 0.05$) than the levels in the patients treated with the coated circuits (group A: 40 ± 13 ng/ml; group B: 54 ± 17 ng/ml at 12 hours after reperfusion).

Discussion

CPB for cardiac operations is often associated with bleeding complications and generation of a systemic inflammatory response, which have been related to systemic heparinization and blood cell activation, activation of the coagulation system, fibrinolytic disorders, and complement activation.^{23, 25, 33-35} The exposure of blood to surfaces in the extracorporeal circuits might contribute to the generalized inflammatory response. Attempts to improve the biocompatibility of the circuits to reduce the systemic reactions have included endothelial cell seeding, heparin-like biomaterials, and heparin surface coating.^{36, 37}

Fig. 2. Circulating plasma levels of IL-8, mean ($\pm$ standard error) in patients who underwent CPB with coated (A, hatched bars) and noncoated (B, cross-hatched bars) circuits. Statistically significant differences ($p < 0.05$) between the groups are indicated with an asterisk. X-clamp, Crossclamp.

Comparison of heparin-coated and uncoated extracorporeal circuits during CPB has shown no significant reduction in platelet activation, fibrinolysis, postoperative blood loss, and donor blood transfusions in routine coronary bypass operations.^{38,39} The only established and consistent clinical effect of heparin-treated circuits is that perfusion can be done with less systemic heparin in selected patients. This may result in improved hemostasis and reduced blood loss and transfusion requirements.⁴⁰ Moreover, a significant reduction of C3 activation products using heparin-coated extracorporeal circuits during CPB has been described.⁴¹ In the present study we demonstrated that the use of Duraflon II heparin-treated extracorporeal circuits reduces the systemic inflammatory reaction during routine CPB procedures as measured by plasma levels of inflammatory mediators.

In agreement with previous studies,^{42,43} increased plasma IL-8 levels in patients undergoing CPB without heparin-coated extracorporeal circuits were observed (see Fig. 2). IL-8, which is produced by monocytes, macrophages, fibroblasts, and vascular endothelial cells after stimulation with TNF- α , IL-1, or lipopolysaccharide,⁴⁴ is considered to be an important and early mediator of neutrophil-mediated inflammatory reactions, because it strongly attracts

neutrophils and enhances their activation. The use of a heparin-coated tubing system showed a remarkable reduction in circulating IL-8 levels. An increase of IL-8 did not occur until 3 to 6 hours after the end of CPB. Because this intervention almost completely prevented early IL-8 production, one may conclude that contact activation might be a major cause of proinflammatory activation in comparison to rewarming, reperfusion, or protamine supply, as has been suggested by Finn and associates.⁴³

Trace amounts of TNF- α were found in a few patients in the present study, whereas in most patients and at most time intervals the TNF- α concentration was below the level of detection. In recent studies similar results were obtained.⁴³ Because TNF- α has a very short half-life in the circulation, frequent serial measurements would be necessary to evaluate the precise role, if any, of TNF- α after CPB. Measurement of TNF- α -induced factors with slower kinetics may offer an opportunity to indirectly assess the role of TNF- α in the inflammatory response after CPB.

TNF- α and inducers of TNF- α have been shown to cause shedding of TNF-R.⁴⁵⁻⁴⁷ Therefore, enhanced levels of sTNF-R may reflect a TNF- α -induced inflammatory response. However, sTNF-Rs were also shown to be dependent on renal func-

Fig. 3. Circulating plasma levels of soluble TNF-R55 (**A**) and TNF-R75 (**B**), ($\pm$ standard error) in patients who underwent CPB with coated (**A**, hatched bars) and noncoated (**B**, cross-hatched bars) circuits. Statistically significant differences ($p < 0.05$) between the groups are indicated with an asterisk.

tion.⁴⁸ In the present study, increases in levels of both sTNF-Rs were found after CPB. Moreover, in patients with heparin-coated circuits the increase of sTNF-R was significantly delayed. Because renal function of the two groups was not different (data not shown), these data suggest that heparin-coating prevents the onset of an early inflammatory response during CPB.

IL-6 is produced by many cell types in response to IL-1 and TNF- α . It may be the main inducer of the acute-phase response to injury, and its concentra-

tion has been shown to be elevated after major operations, reaching peak levels 24 hours after operation.^{49, 50} Here, IL-6 levels peaked as early as 1½ hours after the release of the crossclamp and returned toward normal values within 12 hours in patients treated with uncoated circuits. The underlying reason for these differences in kinetics remains unclear but may be dependent on the kind of trauma, medication, or the presence of neutralizing proteins like IL-6 receptors. Heparin coating of the extracorporeal circuit significantly attenuated sys-

Fig. 4. Circulating plasma levels of sE-selectin, mean ($\pm$ standard error), in patients who underwent CPB with coated (A, hatched bars) and noncoated (B, cross-hatched bars) circuits. Statistically significant differences ($p < 0.05$) between the groups are indicated with an asterisk. X-clamp, Crossclamp.

temic IL-6 levels. Whether this is associated with an attenuated acute-phase response remains to be established.

The soluble adhesion molecule E-selectin increased above baseline starting 3 to 6 hours after crossclamping in both groups, whereas only a modest increase in sICAM-1 was observed 24 hours after CPB. E-selectin is found only on activated endothelium. E-selectin interacts with carbohydrate ligands and mediates the initial rolling of leukocytes on the endothelium. Endothelial cells have been shown to release E-selectin after in vitro stimulation.²⁹ Therefore, specific elevations in levels of sE-selectin would indicate activation or damage to endothelium. The presence of higher levels of sE-selectin at 12 hours after CPB suggests that a more pronounced endothelial inflammatory reaction occurred with the non-heparin-coated circuits. sICAM-1 is constitutively expressed on vascular endothelial cells and a number of other cell types including fibroblasts, epithelial cells, and peripheral blood mononuclear cells and is particularly involved in the firm attachment and transendothelial migration of leukocytes.⁵¹ Its expression is up-regulated after activation during an inflammatory response.

In vitro studies have shown a direct correlation

between the expression of sICAM-1 on endothelial cells and the subsequent shedding of sICAM-1 in the supernatant.²⁹ The ligand for sICAM-1 on the leukocyte surface is CD18. This CD18 expression increases when leukocytes are stimulated and can be used as a sensitive index of leukocyte activation. In a previous study,⁵² increased CD18 expression on neutrophils and concomitant neutropenia after 60 minutes of CPB was found. Heparin coating did not change the CD18 expression and neutropenia. The initial drop in circulating sICAM-1 in patients who underwent CPB with the uncoated circuits suggests an increase in activated leukocytes, because ligand binding by activated leukocytes may clear sICAM-1 from the plasma. On the other hand, CD18-positive leukocytes are immediately trapped by the vascular endothelial cells and are thus withdrawn from the circulation. Immunohistochemical studies are necessary to elucidate this point of discussion.

In conclusion, this study demonstrates that heparin coating of the extracorporeal circuit can significantly reduce the systemic inflammatory reaction caused by CPB. It is conceivable that the heparin-coated surface exerts its protective effect by modifying mediators of the inflammatory cascade rather than from a direct interaction of heparin coating

and coagulation or fibrinolysis. A large clinical study is in progress to provide evidence that postoperative morbidity correlates with proinflammatory activity.

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