Heparin-coated circuits reduce occult myocardial damage during CPB: a randomized, single blind clinical trial

Ali Belboul\textsuperscript{a,b,*}, Christina Löfgren\textsuperscript{b}, Christina Storm\textsuperscript{b}, Marie Jungbeck\textsuperscript{b}

\textsuperscript{a}Department of Cardiothoracic Surgery, University of Gothenburg, Sahlgrenska University Hospital, SE 413 45 Gothenburg, Sweden
\textsuperscript{b}Scandinavian Heart Center, Carlanderska Hospital, Gothenburg, Sweden

Received 9 November 1999; received in revised form 2 February 2000; accepted 9 February 2000

Abstract

Objectives: Cardiopulmonary bypass is associated with a diffuse systemic inflammatory response that can cause considerable morbidity, including organ dysfunction and bleeding. Heparin-coated circuits have been shown to give a reduced inflammatory response with clinical benefits during open-heart surgery. However, the effects on lipid peroxidation, neutrophil activation and myocardial ischemic damage in the human have remained unknown. Methods: In a randomized single blind trial, complement activation, neutrophil counts, malondialdehyde, and cardiac enzymes were studied in 39 patients undergoing open-heart surgery. Two groups were perfused with cardiopulmonary bypass circuits with (n = 20) or without heparin-coating (n = 19). Results: The different complement factors (C3, C4, C3d, C3d/C3 and the C-function), neutrophil levels, MDA and the cardiac enzyme levels were comparable before CPB was started and significantly increased in both groups during bypass. There were significant intergroup differences in the neutrophil levels and MDA after reperfusion (P < 0.0001). Furthermore, significant positive correlations between the lipid peroxidation, expressed as MDA levels, and the levels of neutrophils and the cardiac enzyme, CK-MB were seen within the groups. Conclusions: Heparin coated circuits did lead to a decreased neutrophil response and MDA level. The correlations between CK-MB and neutrophil and MDA levels suggest neutrophil activation leading to lipid peroxidation that may influence myocardial damage. Heparin coating improved biocompatibility and was associated with less occult myocardial ischemic damage in patients undergoing open heart surgery. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Biocompatibility; Lipid peroxidation; Cardiac enzyme; Heart surgery; Heparin coated circuits

1. Introduction

It is well established that blood-material interaction of extracorporeal circuits during heart surgery causes a whole body inflammatory response which includes activation of endotoxin generation, leucocytes, endothelial cells, platelets, the coagulation system, the complement system, and the immune system [1,2]. The release of oxygen free radicals, nitric oxide, endothelins, platelet activating factor, arachidonic acid metabolites, and proteases can activate leukocytes and induce tissue injury. In a final step, the infiltration of leukocytes in ischemic lesions establishes locally an inflammatory response, as in myocardial and cerebral infarctions has been described. This production of inflammatory stimulators and the consecutive leukocyte-endothelial cell interactions are mediated by adhesion molecules (L-selectin, P-selectin, platelet-activating factor, interleukin-8, ICAM-1 and PECAM-1) and shares common pathways with those processes that occur during ischemia/reperfusion injury [3]. Free radical generation facilitates the infiltration of leukocytes into the tissues of the transient ischemic heart after cardiopulmonary bypass (CPB) and produces an inflammatory reaction of the heart. This cardiac inflammation and the occult ischemic damage may play a role in the pathogenesis of postoperative complications like myocardial stunning and coronary low-flow after open-heart surgery. In clinical studies, heparin coated circuits have been shown to decrease the inflammatory response by reducing the activation of biologic cascades [4,5] and decreased the risk of postperfusion injury due to the improved biocompatibility [6,7]. We have shown previously a reduced leucocytosis and blood cell trauma and an improved postoperative morbidity when using heparin-coated circuits [8,9]. Our first concern was the activated neutrophils that migrate through the endothelium and release oxygen free radicals and lysosomal granules resulting in organ damage [10]. This should be controlled if biocompatibility is to be enhanced, and attempts to achieve this have been recently summarized [11]. The purpose of the present study was to investigate if a totally coated circuit system had
a similar reducing effect on complement activation cascades and could alter the postoperative course in a randomized, single blind trial, and if the cellular activation caused by the myocardial reperfusion injury could be reduced by a biocompatible surface giving less myocardial damage.

2. Patients and methods

2.1. Patients

Thirty-nine patients, in stable angina, were prospectively studied in a randomized single blind study and electively operated for coronary artery bypass surgery by using vein grafts and left internal mammary artery. All patients had normal coagulation parameters (platelets, prothrombin complex and bleeding time). Patients with previous cerebro-vascular incidents, diabetes with peripheral vascular complications, bleeding disorders, on anticoagulation therapy, intermittent claudication, pulmonary, renal or hepatic diseases were excluded from the study. The study protocol conformed to the rules of the Helsinki declaration and was approved by the Ethics committee of the University of Göteborg. Informed consent was obtained from patients participating in the study.

Patients were randomized into two groups as described below.

2.1.1. CPB with heparin-coated membrane oxygenator (H)

Twenty patients (all men) were operated with the use of heparin-coated CPB circuits (Durafo II, Bentley/Baxter Inc., Irvine, CA). The mean age was 65.8 ± 6.6 years. Five patients were in NYHA-class II, 15 in NYHA-class III. The mean ejection fraction in the H group was 59.2 ± 14.7%.

2.1.2. CPB with non-coated membrane oxygenator, control group (C)

Nineteen patients (16 males and three female) were operated with non-coated CPB circuits (Bentley/Baxter Inc., Irvine, CA). The mean age was 66.7 ± 9.2 years. Three patients were in NYHA-class II and 16 patients were in NYHA-class III. The mean ejection fraction in the C group was 63.9 ± 13.4%.

2.2. Anaesthesia

Premedication consisted of morphine 0.1 mg/kg i.m. and scopolamine 0.4 mg. Anesthesia was induced with thiopental 3–5 mg/kg body wt., followed by pancuronium 0.1 mg/kg. Fentanyl was given in incremental doses during induction of anesthesia and after intubation up to a total amount of 15 µg/kg. All operations were performed through a midline sternotomy using ECC with cannulation of the ascending aorta and right atrium.

2.3. Cardiopulmonary bypass (CPB)

A Gambro heart-lung machine (Lund, Sweden) with a roller pump was used. The CPB circuit consisted of a membrane oxygenator (BOS-CM50; Bentley/Baxter Inc., Irvine, CA) a venous reservoir (BMR-1900; Bentley/Baxter Inc.) and a cardiotomy reservoir (BCR-3500; Bentley/ Baxter Inc.). The CPB circuit was primed with 2000 ml Ringerdex (Pharmacia, Uppsala, Sweden). A modified St. Thomas solution (Pharmacia, Uppsala, Sweden) was used to achieve cardioplegia and general hypothermia (30–34°C) was instituted. Patients were given the standard dose of systemic heparin (300 IU/kg). The CPB was started when the activated coagulation time (ACT) was above 450 s. Additional heparin was given if the ACT was below 400 s. At the end of CPB, the effect of heparin was neutralized by protamine. Alpha-stat was used for blood gas management. The hematocrit was aimed for 18–25%.

2.4. Measurement of complement activation

The classical complement activation (c-function) was determined by a hemolytic method [9]. Values were expressed as percentage of the activity of pooled normal serum (75–125%). The C3, C4 and C3d fractions were determined by the immuno-electrophoresis technique [9] and values were expressed in g/l or mg/l. Measurements were made at the start of ECC, every 30 min during ECC and at the end of bypass.

2.5. Measurement of routine hematological parameters

Blood samples for total hemoglobin (HGB) white cell count (WCC), platelet count, plasma hemoglobin (P-Hb) and cell differentiation were taken preoperatively, 30 and 60 min of bypass and at the end of CPB, and 1, 3 and 5 days postoperatively.

2.6. Measurement of malondialdehyde (MDA)

Blood samples for assay of plasma MDA as a marker of the free radicals inducing lipid peroxidation (LPO) were taken preoperatively, 30 and 60 min of bypass and at the end of CPB. The blood samples were centrifuged and analysed using the thiobarbituric acid method [12]. LPO in plasma is expressed by MDA (nmol/ml).

2.7. Measurement of cardiac enzyme changes

Aspartate aminotransferase (ASAT) and creatine kinase myocardial-brain isoenzyme (CK-MB) were drawn from the patients preoperatively, and 6, 12, 24 and 72 h postoperatively. The reference values of ASAT and CK-MB in our hospital are < 0.8 µkat/l and < 5 µg/l, respectively. Myocardial infarction was empirically considered when CK-MB level rose over 100 µg/l and ASAT over 2.5 µkat/l with or without ECG changes. Smaller rises in the
enzymes were considered as markers of occult damage due to ischemia and reperfusion.

### 2.8. Postoperative morbidity and complications

The frequencies of postoperative morbidity (postoperative bleeding, respirator usage, blood transfusion, rhythm disturbance, cerebrovascular accidents, and infection) were registered and compared between H and C groups.

### 2.9. Statistical analyses

All means are expressed with one standard deviation (SD). Comparisons between two groups were performed using the Student’s unpaired t-test or the Mann–Whitney test. Serial changes within each group were statistically evaluated by the analysis of variance for repeated measurements. A P-value of less then 0.05 was considered to be significant. Correlations were performed to bring out the association between variables tested.

### 3. Results

There were no significant differences in the mean values for perfusion time, aortic occlusion time, body temperature, oxygen and blood flow rates between the two groups (Table 1).

### 3.1. Complement activation

At the start of CPB, significant ($P < 0.0001$) decreases of the complement fractions C3 and C4 were observed in both groups and remained throughout the operation (Table 2). A concomitant significant time dependent increase of C3d, was observed in both groups, at the start of CPB compared to the preoperative value ($P < 0.001$) and remained throughout the operation (Table 2). Furthermore, the C3d/ C3 quotient, which is less affected by changes of plasma dilution, showed a significant time dependent increase both in the H and C groups (Table 2). The remaining complement function, expressed as a percentage of the hemolytic activity of a pool of normal sera, showed a concomitant decrease due to consumption during CPB in both H and C groups. Compared to the preoperative value, the first significant difference was seen at the start of bypass ($P < 0.0001$) and this remained significant at the end of CPB (Table 2).

When comparing H and C groups, no significant difference was found at any time regarding complement fractions (Table 2).

### 3.2. White blood cells

There was an increase in circulating white blood cells during the operation with a maximum at the end of bypass in both H and C groups ($P < 0.0001$). When comparing the two groups, a significantly higher leucocytosis was seen in the C group ($P < 0.0001$) (Table 3). The increase in white blood cells was caused by an increase in circulating neutrophils, which showed a significant difference between the groups being higher in the C group, ($P < 0.0001$) (Table 3).

### 3.3. MDA changes during ischemia and reperfusion

In the H group, during CPB, the concentration of plasma MDA rose progressively from a preoperative value of $2.67 \pm 0.8$ nmol/ml to $4.56 \pm 1.22$ nmol/ml after 60 min of bypass ($P < 0.0001$) and $6.22 \pm 1.25$ nmol/ml at the end of CPB ($P < 0.0001$) (Table 3). Similar results were seen in the C group.

When comparing the two groups, there were significant differences after 60 min of bypass ($P = 0.0421$) and at the end of CPB ($P < 0.02$), with lower release of MDA in the H group (Table 4).

---

### Table 1

<table>
<thead>
<tr>
<th>Number</th>
<th>H</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.8 ± 6.6</td>
<td>66.7 ± 9.2</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>59.2 ± 14.7</td>
<td>63.9 ± 13.4</td>
</tr>
<tr>
<td>No. of grafts</td>
<td>3.7 ± 1.1</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>ECC-time (min)</td>
<td>80 ± 25</td>
<td>69 ± 16</td>
</tr>
<tr>
<td>AO-time (min)</td>
<td>47 ± 19</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>Rectal temp (°C)</td>
<td>32.2 ± 1</td>
<td>32.4 ± 0.9</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>22 ± 3</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>Heparin (5000 U/ml)</td>
<td>6.7 ± 1.5</td>
<td>6.4 ± 1.8</td>
</tr>
<tr>
<td>Protamine (mg)</td>
<td>317 ± 56</td>
<td>323 ± 48</td>
</tr>
</tbody>
</table>

* ECC, extracorporeal circulation; AO, aortic occlusion.

---

### Table 2

Changes of complement factors in relation to CBT time and heparin (H) or nonheparin (C) coating

<table>
<thead>
<tr>
<th>Time*</th>
<th>C3d (mg/l)</th>
<th>C3 (g/l)</th>
<th>C3d/C3 (mg/g)</th>
<th>C4 (g/l)</th>
<th>C-function (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>C</td>
<td>H</td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>Preop.</td>
<td>3.3</td>
<td>3.8</td>
<td>0.95</td>
<td>0.92</td>
<td>3.6</td>
</tr>
<tr>
<td>30 min</td>
<td>4.1*</td>
<td>4.7*</td>
<td>0.59*</td>
<td>0.56*</td>
<td>7.2*</td>
</tr>
<tr>
<td>60 min</td>
<td>4.6*</td>
<td>5.5*</td>
<td>0.60*</td>
<td>0.57*</td>
<td>8.6*</td>
</tr>
<tr>
<td>End</td>
<td>6.7*</td>
<td>8.0*</td>
<td>0.65*</td>
<td>0.60*</td>
<td>10.0*</td>
</tr>
</tbody>
</table>

*Preop., preoperatively; 30 min, 30 min after the start of cardiopulmonary bypass (CPB); 60 min, 60 min after the start of CPB; end, end of CPB.

*P < 0.001 when the preoperative value is compared to any value within the same group.
3.4. Changes in cardiac enzymes

The mean CK-MB value was significantly raised in both groups from a preoperative value of less then 5 μg/l to 25 ± 14 in the H group and 36 ± 16 in C group 6 h postoperatively (P < 0.001). When comparing the two groups, there were significant difference starting at 6 h postoperatively (Table 5).

The mean ASAT value was significantly raised in both groups from a preoperative value of less than 0.4 μkat/l to 1.1 ± 0.4 in the H group and 1.1 ± 0.3 in the C group 6 h postoperatively (P < 0.001). No significant difference was found at any time between the two groups (Table 5). In both groups the level of CK-MB and ASAT increased significantly compared to preoperative values and remained elevated even 24 h postoperatively (Table 5).

3.5. Plasma hemoglobin (P-Hb)

The mean P-Hb increased significantly with the duration of CPB in both groups (P < 0.0001). The P-Hb in the H group was significantly less elevated when compared to the C group, (P < 0.02) (Table 6).

3.6. Postoperative morbidity

The mean values of postoperative loss of blood via the mediastinal drains and the mean number of blood and plasma units transfused in the various groups showed no significant difference. The mean respirator time showed a similar pattern between the H and C groups. The frequencies of patients developing atrial tachyarrythmias were 4/20 (20%) and 3/19 (16%) for the H and C groups, respectively.

The postoperative morbidity was similar for both groups (Table 7).

3.7. Correlation between neutrophils and MDA

In the heparin coated group, there were significant positive correlations between MDA and neutrophils at the end of CPB (r = 0.632, P = 0.0037) (Fig. 1). In the control group, similar results were found (r = 0.584, P = 0.0138) (Fig. 2).

3.8. Correlation between CK-MB and MDA

There were significant positive correlations between MDA and CK-MB at the end of CPB, (r = 0.595, P = 0.0072), in the heparin coated group. Similar results were seen in the control group (r = 0.524, P = 0.0214).

4. Discussion

This study highlights an enhancement of biocompatibility by heparin-coated ECC circuits on the leukocyte response and oxygen free radical effect on fatty acids thereby reducing occult myocardial ischemic damage in coronary artery bypass surgery. We describe a significant increase in complement activation and leucocytosis with free radical generation during the operation in both heparin-coated and uncoated groups. This could be attributed to the CPB effect on blood components as reported earlier [1]. The significant effects of heparin coating was to reduce leucocytosis, lipid peroxidation and CK-MB release.

Table 5
Cardiac enzyme changes *

<table>
<thead>
<tr>
<th>Time</th>
<th>CK-MB</th>
<th>P</th>
<th>ASAT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H</td>
<td>C</td>
<td></td>
<td>H</td>
</tr>
<tr>
<td>Preop</td>
<td>4 ± 1</td>
<td>3 ± 2</td>
<td>ns</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>6 h</td>
<td>25 ± 14</td>
<td>36 ± 16</td>
<td>0.0123</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>12 h</td>
<td>19 ± 22</td>
<td>26 ± 12</td>
<td>0.04</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>24 h</td>
<td>20 ± 24</td>
<td>18 ± 18</td>
<td>0.038</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>48 h</td>
<td>12 ± 9</td>
<td>13 ± 11</td>
<td>ns</td>
<td>0.7 ± 0.4</td>
</tr>
</tbody>
</table>

* ns, Not significant when comparing H and C groups.
Both groups had similar preoperative and intraoperative characteristics and this could explain their similar clinical postoperative outcome with respect to the duration of ventilation, blood loss, transfusion requirements, myocardial infarction and arrhythmic events.

In a previous publication [8], we had shown that a patient group operated with the heparinized equipment spent a shorter time postoperatively on the ventilator and that this could be related to a reduction in lung damage, resulting in a better condition and oxygenation of the patients. Furthermore, it was shown that the heparin-coated circuits had a significantly lower increase in neutrophil levels, as seen in the present study, but the finding was not emphasized in the pulmonary report.

In this study we also demonstrated increasing levels of leucocytes and MDA during CPB which could support the hypothesis of oxygen free radical damage on the jeopardized myocardium. The main origin of oxygen free radicals during CPB has been credited to activated neutrophils and this is borne out in this study by the strong correlation of MDA and neutrophil generation. Furthermore, the relationship of MDA to CK-MB suggests very strongly that CPB induced white cell damage and free radical generation can damage the myocardium following reperfusion after aortic declamping.

Several mechanisms could contribute to ischaemia and reperfusion injury: The physical occlusion of microvessels

Table 6
Changes in plasma hemoglobin (P-Hb), mg/l

<table>
<thead>
<tr>
<th>Time</th>
<th>H</th>
<th>C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preop.</td>
<td>36 ± 8</td>
<td>32 ± 12</td>
<td>ns</td>
</tr>
<tr>
<td>30 min</td>
<td>82 ± 40</td>
<td>120 ± 57</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td>60 min</td>
<td>190 ± 46</td>
<td>230 ± 69</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td>End</td>
<td>318 ± 103</td>
<td>432 ± 195</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

*Preop., preoperatively; 30 min, after 30 min of extracorporeal circulation; 60 min, after 60 min of extracorporeal circulation; end, end of extracorporeal circulation; ns, not significant. The normal reference interval of P-Hb is less than 50 mg/l.

Table 7
Postoperative morbidity

<table>
<thead>
<tr>
<th></th>
<th>H</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding (ml/24 h)</td>
<td>786 ± 236</td>
<td>765 ± 361</td>
</tr>
<tr>
<td>Atrial tachyarythmias</td>
<td>4 (20%)</td>
<td>3 (16%)</td>
</tr>
<tr>
<td>Respirator time (h)</td>
<td>3.8 ± 1.6</td>
<td>4.4 ± 2.1</td>
</tr>
<tr>
<td>ICU time (h)</td>
<td>38 ± 11</td>
<td>42 ± 17</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>8.2 ± 0.4</td>
<td>8.7 ± 0.8</td>
</tr>
</tbody>
</table>

Fig. 1. A positive correlation ($r = 0.632, P = 0.0037$) was found between MDA, malondialdehyde (nmol/ml) and neutrophils ($10^9$) in the heparin-coated group at the end of cardiopulmonary bypass.
by activated leukocytes which occur under conditions of reduced perfusion pressure where the capillary may behave like a sieve. This trapping phenomenon during ischaemia and reperfusion may become even more pronounced as the release of adhesions molecules lead to increased leucocyte-endothelial interactions [13]. The release of active mediators by activated leucocytes, and granular enzymes, reactive oxygen metabolites and products of membrane phospholipases [14] could contribute to the damage of the myocardium. ECC stimulated neutrophils would also transiently aggregate and adhere to vascular endothelial cells and to myocardial cells after infiltration across the endothelial barrier into the reperfused myocardium [15]. In the presence of the activated complement fragments, activated neutrophils would more easily release their granule contents and generate oxygen free radicals, enhancing the reperfusion injury [14]. These reactive oxygen mediates have been shown experimentally to induce arrhythmias [16] which could be inhibited by free radical scavengers such as allopurinol [17].

The lipid peroxidation pattern suggests a clue to the time when it is mostly generated. When comparing the groups, the first 30 min gives an average rate of a 60% higher production with uncoated circuits, and following this, the generation appears to be stable in both groups with a 5% higher generation in the controls. This finally rises to a 30% higher production, in the controls on reperfusion and rewarming following aortic declamping. This suggests two peak periods of relatively intense free radical generation. The main leucocyte generation appears in the first 60 min and is higher in the controls and then falls at the end of CPB. This suggests that leucocytosis tends to stabilize faster in the heparin-coated group. The reason for this is not known but it can be speculated that the signal mediators of the leucocytosis response are probably made less active in the heparin-coated circuits. Since complement generation was similar in both groups, it is probably the lower production of free radicals and probably other signal substances that modify the intensity of leucocytosis. The cytokines and leukotriines were not monitored in this study. The question arises if heparinized circuits can adsorb such mediators and this needs to be addressed in future studies.

Most studies show that cardiac enzymes do not tend to leak during cardioplegic protection but rather after declamping the aorta. Furthermore, enzyme leakage during CABG without ECC is strikingly absent in the majority of cases suggesting that the long ischemic periods due to global ischemia is a major determinant for reperfusion injury during ECC and aortic clamping. However, the intensity of enzyme leakage was well tolerated in this study in both groups suggesting that...
cardioplegic protection was adequate during the period of cross clamping not leading to significant complications. It is still evident that cardioplegia could not prevent occult myocardial damage with some enzyme loss.

Breda et al. found that reperfusion of isolated ischemic hearts with leukocyte-depleted blood prevented ultrastructural damage and resulted in better myocardial function than when normal blood was used [18]. The sequestration of leukocyte-platelet aggregates in the ischemic heart after declamping [19] has been demonstrated during CPB. The leukocytes are thought to be attracted by free radical generation in the transient ischemic heart after cardiopulmonary bypass giving the occult ischemic damage which may play a role in the pathogenesis of postoperative morbidity after open heart surgery. Lazar et al. showed that the use of heparin-bonded circuits in animals, significantly decreased myocardial ischaemic damage during acute surgical revascularization [20].

In addition, heparin-coated ECC circuits, by reducing neutrophil stimulation, and subsequently free radical release (expressed as increased MDA in this study), was found to reduce occult myocardial damage as reflected by the reduction of myocardial enzyme as seen in this study. This study verifies that the use of the CPB technique is still a potential advantage by using heparin-coated circuits.

References


