In vivo Evaluation of a Phosphorylcholine Coated Cardiopulmonary Bypass Circuit

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ABSTRACT

A complete phosphorylcholine coated cardiopulmonary bypass circuit, including the Dideco D901 oxygenator, was tested for gas transfer, blood path resistance, and biocompatibility in a standardized setting. Blood compatibility was tested by measuring complement and platelet activation.

Three dogs (mean body weight 28 ± 3 kg) were placed on cardiopulmonary bypass at a flow rate of 600 mL/min during 6 hours. The animals were weaned from cardiopulmonary bypass and sacrificed electively after 7 days.

Oxygen and carbon dioxide transfer were 26.6 ± 2.4 mL/min and 33.0 ± 1.9 mL/min, respectively. Mean pressure drop across the oxygenator was 52.6 ± 0.2 mmHg. The respective baseline values for thromboxane B₂, prostaglandin E₂ and platelet factor 4 were 1817 ± 283 pg/mL, 12783 ± 2109 pg/mL, and 0.35 ± 0.08 IU/mL. Thromboxane B₂ and prostaglandin E₂ increased slightly to 2881 ± 868 pg/mL and 18083 ± 3144 pg/mL at 30 minutes of bypass, whereas platelet factor 4 values remained stable curing the procedure. Concentrations of complement split products C5a were only mildly increased.

After use scanning electron microscopy was performed on the inner housing, heat exchanger, and outer surface of the hollow fibers. No thrombi nor organized cellular deposits were found on any of the components. Phosphorylcholine coating of CPB seems to be very promising regarding platelet activation and complement activation.

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INTRODUCTION

Materials used in a cardiopulmonary bypass (CPB) circuit were not originally developed for this application. In general these materials activate the coagulation, complement and fibrinolysis cascades. Together with turbulent flow patterns, zones of blood stasis and the aspiration of shed blood this contributes to the bio-in-compatibility of CPB. In order to reduce this bio-in-compatibility several approaches have been proposed: reduction of foreign surface area, more even distribution of blood flow, avoiding stasis and blood-air interfaces, use of anticoagulant and antifibrinolytic drugs and surface modification of the different materials. An alternative approach is the development of bio-membrane-mimetic surfaces. Such surfaces are designed to mimic the outer surface of blood cells (1, 2). This outer surface is predominantly composed of phospholipid groups, which contribute largely to the non-thrombogenic properties of blood cells. Recent research shows that polymers containing phosphorylcholine reduce protein adsorption and complement activation markedly (3). This study investigates the impact of a complete phosphorylcholine coated CPB circuit on the oxygen transfer, blood elements, coagulation and complement activation.

MATERIAL AND METHODS

The study group comprised three male Labrador dogs with an average weight of 28 ± 3 kg. All animals received care in accordance with institutional guidelines and national laws. A CPB circuit was phosphorylcholine coated from cannula to cannula. The circuit consisted of PVC tubing, a D901 neonatal oxygenator with closed venous reservoir and a venous and arterial cannula. The total priming volume of the circuit was 208 ± 9 mL. The dogs were instrumented, and cannulated via the right carotid artery and right jugular vein. Partial bypass was instituted by means of a roller pump for a 6 hour period at a blood flow of 600 mL/min. The gas to blood ratio was one to one. Before cannulation animals were heparinized with 300 IU/kg body weight. Activated clotting time was maintained above 300 sec during the procedure. Animals were kept at normothermia during the whole procedure.

Pre and post membrane pressures were automatically recorded every 10 sec by means of a Cobe Perfusion Controller connected to a Personal Computer. Arterial and venous blood gases were taken every hour. Red blood cell count, hematocrit, hemoglobin, white blood cell count and formula, platelet count, electrolytes, free plasma hemoglobin, APTT, PTT, fibrinogen, thromboxane B2 (TXB2), prostaglandin E2 (PGE-2), platelet factor 4 (PF4), C5a and TNFα were analysed before instituting CPB and at 30, 60, 120, 240 and 360 minutes of CPB. All values were corrected for haemodilution using following formula:

\[
\text{Corrected value} = \text{measured value} \times \left( \frac{\text{start hematocrit}}{\text{actual hematocrit}} \right)
\]

Haemolysis rate (HR) was calculated using following formula:

\[
\text{HR} = \frac{\text{free plasma hemoglobin (mg/mL)}}{\text{hematocrit}}
\]

Data are expressed as mean value ± standard error of the mean.

At the end of the experiment, the extracorporeal circuit was checked for visible clots and fibrinogen deposits. Electron microscopy was performed on the oxygenator inner housing, heat exchanger, fibers and knots of the weft yarn.

One week after the experiment the animals were sacrificed and autopsy of the lungs, kidneys, and heart was performed.

ANALYSIS TECHNIQUES

Radioimmunoassay was used for the determination of TXB2, PGE-2 and C5a. Platelet factor 4 and TNFα were analysed using enzyme-linked immuno-sorbent assay (ELISA).

DATA ANALYSIS

All data are presented as mean ± standard error of the mean. Statistical analysis using Wilcoxon matched pairs test were applied to compare values of the parameters with their baseline values. Results were significant when p < 0.05.

RESULTS

MASS TRANSFER

Mean oxygen transfer was 26.6 ± 2.4 mL/min (Figure 1). Mean carbon dioxide removal was 33.0 ± 1.9 mL/min.

INLET AND OUTLET OXYGENATOR PRESSURES

Mean inlet and outlet pressure before and after the oxygenator was 160.9 ± 0.3 mmHg and 108.4 ± 0.2 mmHg, respectively. Mean pressure drop across the D901 was 52.6 ± 0.2 mmHg.

HEMATOLOGY AND HEMOLYSIS (TABLE 1)

Platelet count started at a mean value of 159 ± 55/mm3 pre CPB and decreased to a mean value of 123 ± 34 at 30 minutes of CPB, after which it normalized to a mean value of 150 ± 22/mm3 at the end of the experiment (p = NS).

White blood cell count started at 6700 ± 300/mm3, decreased to 5300 ± 200/mm3 at 30 minutes and then steadily

a Dideco, Mirandola, Italy
b Stoßert, München, Germany
c Cobe Cardiovascular, Arvada, Co.
d International Technidyne Corporation, Edison, NJ
e Amersham International, UK
f Perspective Biosystems, USA
g Boehringer, Germany
increased to 12200 ± 1100/mm$^3$ at the end of CPB ($p = 0.01$). Differentiation of the white blood cell count showed no major changes with exception of the eosinophils, which decreased from a baseline average value of 6.3% to 0.3% at the end of the experiment.

Free plasma hemoglobin levels started at a mean value of 63 ± 15 mg/100 mL pre CPB and stabilized at a mean value of 43 ± 11 mg/100 mL at the end of CPB ($p = NS$). Haemolysis rate started at a mean value of 0.26 ± 0.04 mg/mL cells and decreased over time to an average value of 0.10 ± 0.01 mg/mL cells.

**INFLAMMATORY RESPONSE (FIGURE 2)**

C5a levels raised from 14 ± 2.1 IU/mL to 25.9 ± 9.5 IU/mL ($p = NS$) at 30 minutes of CPB after which they returned to baseline values. TNF\(\alpha\) started from a baseline value of 2.8 ± 0.1 pg/mL, then increased to 3.6 ± 1.5 pg/mL ($p = NS$) at 30 minutes, and finally decreased to 2.8 ± 0.9 pg/mL at 360 minutes.

**PLATELET ACTIVATION (FIGURE 3)**

Thromboxane B2 and PGE-2 levels increased from a starting value of 1817 ± 283 pg/mL and 12783 ± 2109 pg/mL respectively, to 2881 ± 868 pg/mL ($p = NS$) and 18083 ± 3144 pg/mL ($p = NS$) at 30 minutes, after which the levels returned to 1595 ± 353 pg/mL and 9897 ± 3175 pg/mL at the end of CPB. Platelet factor 4 and LDH values remained stable during the experiment.

**AUTOPSY**

Autopsy of heart, lungs and kidneys did not reveal any pathologic lesions in the dogs.

**SCANNING ELECTRON MICROSCOPY**

Examination of the polycarbonate housing (Figure 4), the stainless steel heat exchanger (Figure 5), polypropylene fibers (Figure 6) and knots of the weft yarn (Figure 7) showed almost no deposition of proteins and platelets.

**DISCUSSION**

Although phosphorylcholine coatings have already been applied with good results on chest tubes and coronary stents they have never been used in a complete CPB circuit (4, 5). Coating of microporous hollow fibers can cover the micropores with a small layer of coating what can result in a higher resistance to diffusion. The application of a small layer of phosphorylcholine on the gas exchange fibers does not influence the oxygen transfer of the oxygenator (Figure 1). Scanning electron microscopy photographs of the hollow fiber showing open pores support this finding (Figure 6). The obtained oxygen transfer data are not only comparable with the ones provided by the manufacturer but also show a high reproducibility amongst the different oxygenators.

Whole body inflammatory response to CPB is highly complex, and complement appears to be just one component. The alternative complement pathway is activated during CPB and

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**Table 1. Evolution of hematocrit, leukocyte count, white blood cell differentiation, platelets, free plasma hemoglobin. Values are expressed as mean ± standard error of the mean**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>360</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>39.7 ± 2.8</td>
<td>37.4 ± 2.7</td>
<td>37.0 ± 2.3</td>
<td>41.8 ± 4.1</td>
<td>30.8 ± 2.8</td>
<td>31.5 ± 1.8</td>
</tr>
<tr>
<td>WBC (×1000/mm$^3$)</td>
<td>6.7 ± 0.3</td>
<td>5.3 ± 0.2</td>
<td>6.2 ± 0.5</td>
<td>7.4 ± 0.5</td>
<td>10.6 ± 0.7</td>
<td>12.2 ± 1.1</td>
</tr>
<tr>
<td>Segmented (%)</td>
<td>68.7 ± 1.2</td>
<td>71.0 ± 2.1</td>
<td>74.3 ± 2.4</td>
<td>77.3 ± 2.4</td>
<td>83.3 ± 1.3</td>
<td>84.7 ± 0.9</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>20.7 ± 0.9</td>
<td>18.7 ± 1.9</td>
<td>16.0 ± 1.0</td>
<td>13.7 ± 1.5</td>
<td>12.0 ± 1.5</td>
<td>11.3 ± 0.3</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>4.0 ± 1.7</td>
<td>5.3 ± 0.9</td>
<td>5.3 ± 1.2</td>
<td>6.0 ± 1.5</td>
<td>3.7 ± 1.7</td>
<td>3.7 ± 1.2</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>6.3 ± 0.9</td>
<td>5.0 ± 0.0</td>
<td>4.3 ± 0.7</td>
<td>3.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Platelets (×1000/mm$^3$)</td>
<td>159 ± 55</td>
<td>123 ± 34</td>
<td>127 ± 31</td>
<td>121 ± 30</td>
<td>132 ± 28</td>
<td>150 ± 22</td>
</tr>
<tr>
<td>Free plasma hemoglobin (mg/100mL)</td>
<td>63 ± 15</td>
<td>66 ± 27</td>
<td>81 ± 29</td>
<td>58 ± 21</td>
<td>54 ± 20</td>
<td>43 ± 11</td>
</tr>
</tbody>
</table>
results in the activation of C5 to C5a and C5b. C5a activates neutrophils and C5b initiates the formation of the membrane attack complex, which is capable of producing cell lysis and death (6). Whereas complement levels up to four times the baseline are observed during CPB (7, 8), during our experiment only a relatively small increase in C5a level of 46% is noted at 30 minutes of bypass. During CPB, leukocyte count first decreases, in response to hemodilution, after which it increases moderately during the procedure. At the same time monocytes and neutrophils are activated, while lymphocytes count decreases resulting in a higher susceptibility to infection postoperatively (6). Our results show a comparative evolution in white blood cell count, but no major changes in white blood cell differentiation with exception of the eosinophil count. Although no real markers for neutrophil activation, such as Mac-1, were measured, one could speculate on less activation of neutrophils due to the lower generation of C5a. This is in line with previous research (9).

In uncoated circuits platelet activation is expressed by an increase of both thromboxane B2 and PGE-2 up to 4 times the baseline (7, 10). In our study only a mild activation of platelets is observed at 30 minutes of bypass, but the overall activation by the surface is much lower as shown by the constant values of thromboxane B2, platelet factor 4, PGE-2 and LDH. This suggests that a phosphorylcholine coated CPB has excellent non-thrombogenic characteristics.

Free plasma hemoglobin levels and hemolysis rates show values comparable with those reported for uncoated circuits (7).
When heparinized blood comes into contact with nonendothelial surfaces, plasma proteins are instantaneously adsorbed onto the surface. All these nonendothelial surfaces produce a thrombotic stimulus, but the stimulus seems to vary between surfaces. Heparin-bound surfaces seem to be more thromboreistant. This study, in agreement with the literature, shows that phosphorylcholine coated surfaces are at least equal in thromboreistance as is shown by the SEM analysis of housing, fibers, heat exchanger and weft yarn. However, in contrast to heparin bound surfaces, which lose their antithrombotic properties after exposure to protamine, phosphorylcholine coating can be expected to resist to contamination of the circulating blood with protamine (11).

Phosphorylcholine coating of CPB seems to be very promising regarding platelet activation and complement activation, which makes it a full alternative for heparin bound surfaces. However, these promising results should be confirmed by expanding the series. Clinical studies should clarify if these results can be reproduced during cardiac surgery with a certain degree of organ ischemia and reperfusion, which are both known to activate complement and platelets.

REFERENCES