Granulocyte Elastase Release during Extracorporeal Oxygenation: Effects of Bubble, Membrane, and Hollow Fiber Oxygenators

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Abstract

Activation or lesions of PMN-granulocytes among other effects result in release of proteases i.e., elastase (E). Plasma E is mainly inactivated by alpha 1-proteinase inhibitor (alpha 1-PI) by formation of E-alpha 1-PI complexes. However, E may be able to destroy plasma proteins (i.e., clotting factors, immunoglobulins, etc.) before complex formation occurs. The effects of extracorporeal oxygenators on blood cells were investigated in samples of 26 patients before, during and up to three days after extracorporeal circulation (ECC) and oxygenation in open heart surgery. Three types of oxygenators were compared in clinical use: bubble (A), membrane (B), and hollow fiber (C). A significant increase of E alpha 1-PI concentrations is observed during ECC. The extent of E release depends on time of ECC and on the type of extracorporeal oxygenator (E alpha 1-PI levels at the end of ECC: 1514 ± 557 ng/ml –A, 842 ± 480 mg/ml –B, 506 ± 128 ng/ml –C). Complex levels increase further on and reach maxima 2h after the end of ECC. The increase of E in plasma is more pronounced than other hemolytic parameters. E-alpha1-PI complex levels thus seem to be a sensitive indicator for leukocyte lesions and/or activation during extracorporeal oxygenation.

Introduction

Three different types of extracorporeal oxygenators are in clinical use nowadays: bubble-, plate membrane-, and hollow fiber oxygenators. Though extracorporeal circulation (ECC) and oxygenation have been proven to be safe and uncomplicated in open heart surgery, deleterious effects on various blood components are observed using any of these oxygenators.1 It was found that the destruction of red blood cells seems to be dependent on time of ECC and the type of oxygenator.2 Other investigators found that postoperative complications (i.e., bleeding, infections, respiratory distress) are due to thrombocytopenia,3 neutropenia4 and/or complement activation5 during ECC.

In patients requiring chronic hemodialysis lesions or activation of polymorphnuclear (PMN) granulocytes are observed due to contact of blood components to dialyser membranes, resulting in release of the neutral protease elastase.6 Plasma elastase is readily inactivated by the proteinase inhibitors alpha 1-proteinase inhibitor (alpha 1-PI) and alpha-2-macroglobulin.7 However, an imbalance of released elastase and inhibitor concentration may lead to proteolytic degradation of various plasma proteins.7 Thus elastase was shown to be able to destroy components of connective tissue (i.e., proteoglycans, various collagens, fibronectin, elastin), factors of the complement system (C3 and C5), proteinase inhibitors (i.e., antithrombin III, alpha 2-plasmininhibitor, Cl-inactivator) and carrier-proteins like transferrin and prealbumin.8

In this study the effects of three different types of extracorporal oxygenators on blood cells and on plasma proteins are investigated.

Materials and Methods

Three groups of extracorporeal oxygenators were compared in clinical use: bubble oxygenator (n = 5) Optiflow II; membrane oxygenator (n = 15), CML and
Blood samples of 26 adult patients undergoing routine cardiac surgery were examined. Samples were taken before, during and up to three days after ECC in open heart surgery. Distribution of oxygenator to patient and kind of surgical procedure was randomized. The time of ECC and the sex and age of patients were comparable in all groups, as was the postoperative treatment, duration of assisted ventilation after the operation, medical treatment, and other technical items.

All values listed and shown were corrected for hemodilution during and up to two hours after ECC according to the formula: value of parameter × initial hematocrit/hematocrit at according ECC-time. EDTA-blood samples were analyzed on H 6000.

Plasma levels of elastase alpha 1-PI complexes were determined by an enzyme linked immunoassay. Complement components of C3c and C4 were quantified by laser-nephelometry using antisera.

Results

Plasma hemoglobin concentrations indicating red blood cell lesions show time dependent increase during extracorporeal circulation and reach maxima 90 minutes after onset of cardiopulmonary bypass (Figure 1). Beginning with the first postoperative morning a stabilization at nearly initial values is observed. After the onset of bypass in membrane and hollow fiber oxygenators platelet counts decreased to about 80% of initial values (Figure 2). Bubble oxygenator patients, however, showed drastically reduced platelet counts at the end of bypass (about 50% of initial counts) and had lower platelet counts in the post operative days (results not shown).

Leukocyte counts in all three oxygenator groups are nearly constant during ECC. Independent from oxygenator type at the end of bypass leukocyte counts increase to about 200% of initial counts and reach maxima two hours after the end of bypass (Figure 3a). The relative amount of neutrophils is reduced from the beginning of ECC. Whereas in membrane and hollow fiber oxygenators 90 minutes after the onset of bypass an increase to initial values is observed, neutropenia in the bubble oxygenator group persists till the end of ECC (Figure 3b).

Plasma levels of elastase alpha 1-PI complexes do not correlate with the corresponding total leukocyte counts. Complex levels increase depending on time during ECC and—90 minutes after the beginning of bypass—they reach a first maximum. A slight decrease at the end of ECC is followed by a further increase to maxima two hours after ECC (Figure 4). In the bubble oxygenator group at the end of ECC the highest elastase complex levels are measured (1514 +/− 557 ng/ml). In contrast to bubble oxygenators membrane and hollow fiber oxygenators caused a 2.5- and 4-fold

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b Travenol, Deerfield, IL 60015
c Terumo, Piscataway, NJ 08854
d Technicon, Tarrytown, NY 10591
e Merck Company, Darmstadt, Federal Republic of Germany
f Hoechst-Behring AG, Marburg, Federal Republic of Germany
increase of complex levels to $842 +/\text{1} 480 \text{ng/ml}$ and $506 +/\text{1} 128 \text{ng/ml}$, respectively.

Consumption of complement factors was observed during ECC (Table 1). Whereas C4 complement shows a very similar decrease to 60–75% of initial values in all oxygenator groups, there are significant differences in consumption of C3 complement (Figure 5).

Table 1
Concentrations of C3c and C4 complement at the end of extracorporeal circulation and oxygenation in open heart surgery (mean $+/\text{1}$ s.d.). Preoperative values are listed in parenthesis.

<table>
<thead>
<tr>
<th>Oxygenator</th>
<th>C3c</th>
<th>C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bubble oxygenator</td>
<td>0.562 +/\text{1} 0.248</td>
<td>0.201 +/\text{1} 0.125</td>
</tr>
<tr>
<td>Membrane oxygenator</td>
<td>0.766 +/\text{1} 0.121</td>
<td>0.238 +/\text{1} 0.099</td>
</tr>
<tr>
<td>Hollow fiber oxygenator</td>
<td>1.126 +/\text{1} 0.282</td>
<td>0.334 +/\text{1} 0.045</td>
</tr>
</tbody>
</table>

Figure 3: Changes of total leukocyte counts (a.) and neutrophils (b.) during and after extracorporeal oxygenation. Bubble oxygenator (--), membrane oxygenator (········), hollow fiber oxygenator (---).

Figure 4: Release of granulocyte elastase during and after extracorporeal oxygenation. Plasma levels of elastase in complex with alpha 1-proteinase inhibitor were determined by enzyme linked immuno assay/bubble oxygenator (--), membrane oxygenator (········), hollow fiber oxygenator (---).

Figure 5: Consumption of complement during extracorporeal oxygenation. Percent of initial C3c and C4 concentrations at the end of ECC are shown.
Discussion

In this study the deleterious effects of extracorporeal oxygenators on blood cells were investigated. It was shown that cell lesions are dependent on time of extracorporeal circulation and on type of oxygenator. Like other investigators we observed a stronger erythrocyte damage by bubble oxygenators than by membrane and hollow fiber oxygenators. Platelet counts in bubble oxygenator decreased more drastically than in the other tested oxygenator types as well. This stronger decrease of platelet counts may be due to the contact of blood to the mesh necessary for defoaming. Since stimulated granulocytes are known to destroy plasma proteins and/or are able to induce endothelial cell damage by release of toxic oxygen metabolites, the effects of extracorporeal oxygenators on leucocytes are of particular importance. In our study we could show leukocyte stimulation during extracorporeal circulation by the determination of elastase in complex with alpha 1-proteinase inhibitor. Accordingly, stimulation is dependent on time of ECC and on type of oxygenator. In hemodialysis the release of PMN-granulocyte elastase is due to contact of blood cells to membrane surfaces resulting in so called frustrane phagocytosis. Since in hollow fiber oxygenators, with a capillary surface of more than 5 m² the increase of complex concentrations is even lower than in membrane oxygenators, elastase release can not only be due to contact of blood cells to foreign surfaces. Chenoweth et al. could show that oxygen/blood contact may result in granulocyte stimulation by activation of the complement system. Accordingly the complement derived anaphylatoxins C3a and C5a activate granulocytes and account for "post-pump syndromes." In hemodialysis pulmonary dysfunction could be due to complement activation. Since we could show that consumption of complement, just like granulocyte stimulation, is dependent on the type of oxygenator, this effect may be due to complement activation as well.

The particular importance of elastase release which reflects the activation of granulocytes during extracorporeal oxygenation is to be seen in Figure 6. The short time of extracorporeal circulation (in this example 15 minutes) causes only poor granulocyte activation. On the other hand longer times of ECC and high initial leucocyte counts due to excessive granulocyte stimulation and in this particular case probably caused an apoplectic fit.

References


Figure 6: Release of granulocyte elastase (a.) and changes of leukocyte counts (b.) during and after extracorporeal oxygenation with a bubble oxygenator. (-) patient A, (-----) patient B—15 min. bypass time, (-----) medium values of 10 patients.

**Question from the Audience**

*Question—Sally Wallins:* Can you tell me specifically what bubble oxygenators are used?
*Answer:* In the last experiments, I think it was a Shiley one. But I don’t know exactly, because I’m not a perfusionist. I’ve been told by the perfusionist.