Synthetic protein treated versus heparin coated cardiopulmonary bypass surfaces: similar clinical results and minor biochemical differences

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Abstract

Objective: Complications associated with cardiopulmonary bypass (CPB) have gained more attention due to increased interest in coronary artery bypass grafting without CPB. The impact of heparin coating of CPB circuits has been discussed controversially. The present study examines if the treatment of the oxygenator surface with a synthetic protein may serve as an alternative to a completely heparin coated circuit.

Methods: Fifty-eight patients undergoing coronary artery bypass grafting with CPB were randomly assigned to completely heparin coated circuits or synthetic protein treated oxygenators in a double blind protocol, focusing on the inflammatory reaction resulting in membrane damage, coagulation changes and markers of cerebral injury or dysfunction. Treatment groups did not differ as to preoperative demographics, and intraoperative clinical data. Patients with any neurologic disease or risk factors for cerebral dysfunction were not included in the study.

Results: Postoperative clinical data did not differ between groups. Both surface treatments resulted in similar coagulation activation, hyperfibrinolysis and disseminated intravascular coagulation. Platelet count displayed a difference in favor of the heparin coated group ($P \leq 0.029$). Increased leukocyte activation reflected by rising myeloperoxidase concentrations on CPB was present in both synthetic protein and heparin coating groups. Interleukins 6 and 8 reacted similarly, but interleukin 8 increased significantly more ($P \leq 0.0061$) at the end of surgery in patients treated with protein treated oxygenators. The same pattern was observed for complement activation as determined by total complement complex ($P \leq 0.006$). Both surface changes resulted in moderately increased S-100B protein and neuron specific enolase, without difference between groups. Both markers did not reach concentrations associated with clinical manifestation of cerebral injury.

Conclusions: These results in routine patients with short bypass time, imply that protein treated oxygenators are associated with a limited increase of biochemical markers similar to heparin coating. However, significantly lower interleukin 8 release and complement activation can be achieved by heparin coating. The protein treatment is a standard feature of the oxygenator examined in both groups. It is not associated with additional cost and therefore appropriate for use in routine patients. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

After 45 years of worldwide clinical experience with cardiopulmonary bypass (CPB) [1], this technology remains a prerogative for most cardiosurgical procedures. The pathogenicity of CPB has been continuously reduced by numerous minor and major advances throughout the years [2]. The remarkable safety level that has been achieved, is currently challenged by a trend towards the reduction of surgical trauma by lesser invasive surgical techniques [3], which may gradually demask even minor complications associated with CPB.

The whole body response to CPB may lead to organ dysfunction [4], and cause temporary biochemical disorders in every patient. Due to major advances in oxygenator technology [5], current research focuses on the biocompatibility of CPB [6], and specifically on reducing the pathogenicity of CPB surfaces by coating [7] or modification [8]. Additional technologies such as leukocyte or particle filtration are evolving concepts to prevent CPB-related pathogenicity.

The historical perspective for the development of heparin coating technologies is: (1) Possible reduction or avoidance


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Liquemin™ (Roche, Grenzach-Wyhlen, Germany). The study underwent institutional review, and was approved by the institution’s ethical committee (No. 102/96).

2. Methods

2.1. Patients

Fifty-eight consecutive patients, who underwent CABG between March and July 1997 at the Department of Thoracic and Cardiovascular Surgery at the J.W. Goethe University, were included in a prospective double-blind randomized trial after giving informed written consent. The study underwent institutional review, and was approved by the institution’s ethical committee (No. 102/96).

Very strict exclusion criteria were followed, to ensure that potential clinical and biochemical effects would be associated with CPB surface technology rather than comorbidity (Table 1).

Patients were randomly assigned to two groups (39 patients in group A, 19 in group B): in group A, a protein treated oxygenator was used, in group B CPB was performed with a heparin coated system, including oxygenator and tubing set. All patients were oxygenated with a Quadrox™ oxygenator (Jostra AG, Hirrlingen, Germany).

Demographic data of the two patient groups are listed in Table 2.

2.2. Cardiopulmonary bypass

After anticoagulation by systemic administration of heparin (300 IU/kg Liquemin™ (Roche, Grenzach-Wyhlen, Germany)), CPB was instituted with a Quadrox™ capillary membrane oxygenator and tubing set, including an arterial filter (40 μm; Pall, Dreieich, Germany). The circuit was primed with 1500 ml Ringer’s lactate, 500 ml 6% hydroxyethyl starch (HES), 100 ml 20% mannitol, and 150 U/kg of heparin (Liquemin™) using a prebypass filter (Pall, 0.2 μm, Dreieich, Germany). In patients with serum creatinine above 1.5 mg/dl, 500 ml Ringer’s lactate was used instead of HES. Additional heparin was administered when activated clotting time (ACT) fell below 400 s. A flow of 2.4 l/min per m² body surface was applied. Rectal temperature was lowered to 32 ± 2°C. The left ventricle was vented through the cardioplegic needle in the ascending aorta. Proximal anastomoses were performed after release of the aortic crossclamp using a side biting clamp. Heparin was fully antagonized with protamine sulphate at the end of CPB. Antegrade cold blood cardioplegia was used in an intermittent fashion and reinfusions were performed every 20 min. The arresting dose was 1000 ml and the maintenance dose amounted to 400 ml.

Anesthesia was maintained with sufentanyl, pancuronium or propofol, ventilation was performed with a O₂/N₂O...
mixture establishing a FiO2 of 0.5 and aiming at a pCO2 of 35–40 mmHg. Chest drain blood loss during the first 24 h, application of catecholamines for inotropic support, as well as the need for packed red cells and fresh frozen plasma (FFP) were documented as clinical end points.

CK and CK-MB, troponin T and myoglobin were monitored postoperatively to assess myocardial protection. The platelet count was determined, and to show impairment of the coagulation cascade or disseminated intravascular coagulation antithrombin III (AT III) and fibrinogen, D-dimers, and prothrombin fragments F1 + 2 (F1 + 2) were assessed. The plasmin-α2-antiplasmin complex (PAP) served to quantify hyperfibrinolysis.

To assess the inflammatory whole body response, interleukin 6 and 8 (IL-6, IL-8; ELISA, R&D Systems, Minneapolis, MN) were determined. IL-6 in CPB reflects pulmonary and cardiac dysfunction. Raised IL-6 levels have been reported to correlate with post-CPB left ventricular wall-motion abnormalities and myocardial ischemic episodes, whereas IL-8 is primarily associated with cardiovascular dysfunction. IL-8 is produced in response to a wide variety of proinflammatory stimuli such as exposure to IL-1, TNF, LPS and viruses. It is a potent chemoattractant for neutrophils. In addition, IL-8 also has a wide range of other proinflammatory effects. IL-8 causes degranulation of neutrophil specific granules and azurophilic granules, enhances the adherence of neutrophils to endothelial cells and sub-endothelial matrix proteins [13]. C5b9 was employed reflecting the final step of complement activation.

Human myeloperoxidase (MPO) is a hemoprotein with a molecular weight of 140 kDa. MPO is stored in primary granules (azurophilic) of neutrophils. It is a major component of the bactericidal armamentarium of neutrophils, due to its capacity to catalyze the production of hypochlorous acid (HOCl), a powerful oxidant that is derived from chlorine ion (Cl−) and hydrogen peroxide (H2O2). In a number of inflammatory situations, MPO is released in the extracellular medium where its measurement can be used as an index of neutrophil activation, a major source of reactive oxygen intermediates leading to membrane injury. It was determined with an enzyme-linked immunoassay (MPO-ELISA; R&D Systems, Minneapolis, MN).

Arterial blood samples were collected before induction of anesthesia, when CPB was installed, immediately prior to and 5 min after release of the aortic crossclamp, 1 h, 24 h, and 5 days after surgery. Concentrations were not adjusted for the hemodilution caused by the circuit prime. This resulted in the potential masking of an increase in certain variables at the onset of CPB.

S-100B protein and NSE served to quantify cerebral injury biochemically. The S-100B protein was determined using a sensitive luminometric assay (Byk-Sangtec, Lund, Sweden), which selectively measures the beta subunits (present in glial and Schwann cells), as opposed to the alpha subunits (present in striated muscle, heart and kidney). We determined NSE in serum specimens with an ELISA test (Boehringer Mannheim Immundiagnostica, Mannheim, Germany), where 95% of healthy individuals show serum concentrations below 13 μg/l (detection limit 0.5 μg/l).

2.3. Statistical analysis

Statistical analysis was carried out using the SAS software package. The Mann–Whitney U-test was employed to compare differences between the two groups in the absence of a normal distribution, and ANOVA for repeated measures was employed to assess increases of variables over time. Separate ANOVAs were used for each group. Bonferroni corrections were used for post hoc comparisons. Data are presented as mean ± standard error of mean. Differences were considered significant, when the P value was below 0.05.

3. Results

3.1. Intra- and postoperative data

Bypass time, aortic crossclamp time, lowest rectal temperature, and prebypass and on bypass heparin dosage did not differ between groups (Table 2). In the postoperative period, the time on mechanical ventilation did not show a difference between polypeptide coated and heparin coated surfaces, and both patient groups suffered a similar amount of blood loss. One patient in the heparin coating group with a history of severe obstructive lung disease died on postoperative day 4 of pulmonary failure associated with SIRS. There was no mortality in the polypeptide treatment group. Four out of 38 polypeptide treatment patients, and three out of 19 heparin coating patients temporarily required inotropes after the operation, and two (group A) versus zero patients (group B) required reexploration for bleeding. The mean amount of transfused packed red cells and fresh frozen plasma did not differ in relation to surface changes (Table 3).
3.2. Hemostaseologic results

Both surface treatments resulted in a similar degree of coagulation activation, hyperfibrinolysis and disseminated intravascular coagulation (Table 4). There was a significant difference in platelet count in favour of heparin coating ($P = 0.029$).

3.3. Systemic inflammatory reaction

There was clear evidence of increased leukocyte activation reflected in rising myeloperoxidase concentrations on CPB in both groups, without difference between protein treatment and heparin coating (Fig. 1). IL6 and IL8 (Fig. 2a,b) reacted in a similar fashion, but IL8 was significantly higher in group A at the end of surgery. Accordingly, a significant difference was observed for complement activation as determined by membrane attack complex (C5b9, Fig. 3).

3.4. Cerebral markers

Both surface treatments resulted in a moderate increase in S-100B protein (Fig. 4a), as well as NSE (Fig. 4b). Both markers did not reach concentrations indicating significant cerebral injury.

3.5. Myocardial ischemia markers

CK, CK-MB, and troponin T values showed similar myocardial protection in both groups. Myoglobin concentrations reflecting muscle injury did not differ significantly between groups (Table 3).

4. Discussion

The contact of blood with foreign surfaces in oxygenators, tubing and filters is one of several contributors to the multifactorial pathogenicity of CPB, with blood gas interaction and cardiotomy suction potentially playing an even more important role [6]. The current study was designed to assess two approaches to surface modification and/or coating, which differ in their impact on biochemical outcome variables.

Since the first efforts by Gott [14] to attach heparin to CPB surfaces, several heparin coating technologies have been developed, to achieve a possible reduction of anticoagulation for CPB [15]. A second rationale behind heparin coating of CPB circuits or oxygenators alone is to reduce the interaction between blood and foreign surfaces [16], which involves modified adsorption of plasma proteins to the surface. This creates an inactive surface with reduced
attachment of fibrinogen resulting in lower adsorption and activation of platelets [17]. Furthermore, the continuous formation of a complex between ATIII and thrombin is catalyzed. A third rationale behind heparin coating is a reduction of the risk of oxygenator failure, which can be achieved with both surface treatments on the Quadrox™ oxygenator employed in both groups of the present study. The surface treatment, or coating, is an additional improvement of an oxygenator with a very small pressure gradient of 39 mmHg without, and 32 mmHg with Bioline™ coating [18].

The biochemical benefit of heparin coated CPB circuits has been shown by several researchers in animal models [19] and in clinical studies. An important difference between various clinical studies is the lower [5], or equal ACT level [12] for heparin coated versus uncoated CPB circuits. When using different ACT levels in study groups, one cannot distinguish between the effects of heparin coating alone, and those of reduced anticoagulation. Other authors could not confirm some of the postulated benefits of heparin coating, particularly that heparin attached to the blood contact surface may limit complement activation [20] or change beta thromboglobulin or fibrinopeptide A levels [21].

The treatment of artificial surfaces with synthetic proteins may be an alternative to heparin coating, because it is capable of avoiding the adsorption of blood cells on the oxygenator surface. The original idea behind using the synthetic protein treatment, was to prevent the high pressure drop phenomenon, a rise in pressure difference between the inlet and outlet of membrane oxygenators, probably due to a pathologic fibrin layer increasing the pressure gradient across the membrane.

The high standard of current CPB systems has made it increasingly difficult to test technical improvement in clinical studies involving relatively small patient groups. In most cases, the statistical power of such studies will not suffice to show a significant clinical benefit associated with changes in the CPB circuit. The complications that become clinically evident represent merely the tip of the iceberg, whereas the large ice mass under the water represents those laboratory findings that have not become clinically relevant. For the future, there is the choice to rely on laboratory outcome variables, which is the more unsatisfactory option, or to study larger patient groups to achieve the statistical power to reveal a clinical benefit. Another option pursued in the current protocol is a strict patient selection regarding concomitant disease and risk factors for CPB related complications.

The biochemical effects of heparin coating versus untreated surfaces have been shown previously [22] by comparing untreated oxygenators to otherwise identical heparin coated circuits and oxygenators. The coated circuits were associated with reduced leukocyte and complement activation, and less cerebral and/or blood brain barrier changes. In the relatively small number of routine CABG patients studied, a clinical benefit for heparin coating was not evident. Other authors have demonstrated that oxygenators and circuits heparin coated with various technologies, lead to biochemical, but not clinical improvement [23].

The study by Steinberg et al. [24] assessed interleukins 1, 2, 4 and 6, and found an increase of IL6 due to lung dysfunction. Increased production of interleukin 6 has been associated with post-bypass lung dysfunction and impaired hemostasis in additional studies, and IL8 levels correlated with post-bypass cardiovascular dysfunction [25]. We there-
fore chose those two cytokines for the current study, and observed a significant increase of both variables after CPB. IL8 was markedly lower with heparin coating, but did not correlate with post-bypass cardiovascular dysfunction. The different reaction pattern of these cytokines is open for speculation, because of the similar mechanism of their activation.

Leukocyte activation, as determined by myeloperoxidase release, showed a similar time course in both groups (Fig. 1), demonstrating the need for further control of activated leukocytes by prevention of activation and/or filtration of these cells. The amount of complement activation, as measured by membrane attack complex, differed significantly between groups after release of the aortic crossclamp (Fig. 3), indicating a possible effect of surface bound heparin on complement activation. The interaction between complement and leukocyte activation would suggest a reduced myeloperoxidase release in heparin coating patients, which was not observed. Therefore, other mechanisms of complement activation may be involved in the reduced release of C5b9 in group B.

Postoperative platelet count was reduced in all patients examined, showing a significant difference between groups 1 h postoperatively ($P = 0.029$; Table 4), reflecting a platelet sparing effect due to decreased platelet adhesion to heparin coated surfaces. The degree of disseminated intravascular coagulation as measured by AT III, and fibrinogen was identical in both groups, which was also true for D-dimers, F1 + 2, and PAP, representing coagulation activation and hyperfibrinolysis associated with CPB. These domains are often considered among the variables affected by IV heparin. The lack of changes between groups suggests that surface bound heparin does not significantly alter the interference of IV heparin with coagulation and fibrinolysis. Based on the present data, the quality of extracorporeal surfaces in contact with blood is not the dominant factor determining the activation of the coagulation cascade.

S-100B protein has become a frequently used marker of cerebral injury and/or blood brain barrier dysfunction. Serum concentrations react rapidly not only to neuronal injury, but even transient and clinically irrelevant changes in blood brain barrier permeability lead to increased S-100B release [26]. Healthy individuals show serum concentrations below the detection limit of 0.2 mg/l. Drawbacks of this approach is the current standard treatment (unpublished data), need to be considered. The current double-blind protocol allowed us to eliminate this problem, so that at least the differences between groups supply reliable information. As opposed to previous studies comparing S-100B between heparin coated and untreated CPB circuits/oxygenators, the difference between protein treatment and heparin coating was not significant before, during and after surgery.

NSE is found predominantly in neurons and its release is a marker of outright neuronal damage, such as in TIA, stroke, or intracerebral hemorrhage. Concentrations correlate with the volume of the lesion [27]. It is a sensitive indicator of even small brain infarcts [28].

Our results imply that heparin, as a pharmacological agent, may not be the only source of improvement associated with heparin coating. We assume that the synthetic protein treatment contributes significantly to improved biochemical results, when compared to previous studies, where completely untreated surfaces served as controls [24].

We conclude, that both polypeptide coating and heparin coating are safe and effective surface treatments for CPB. Both comprise an improvement as compared to CPB circuits and oxygenators examined previously. However, the polypeptide coating approach is the current standard treatment of the Quadrox™ oxygenator, and is not associated with additional cost. Safeline™ is therefore appropriate for use in routine patients. In patients undergoing long-term extra-corporeal support or ECMO, the advantages of heparin coating may become more evident.

References


Appendix A. Conference discussion

Professor K. Taylor (London, UK): In relation to the free radical-induced injury that you are talking about, do you think that is mediated through leukocyte activation, and do you have any data on either the increase in neutrophil numbers or neutrophil trapping in the lung in your patients?

Dr Wimmer-Greinecker: I don’t have any data on the neutrophil numbers, no.

Dr L. von Segesser (Lausanne, Switzerland): We had heard in the postgraduate course on Sunday from the Regensburg group, that there was a significant reduction in the transoxygenator gradient for coated oxygenators. Can you confirm this?

Dr Wimmer-Greinecker: We haven’t looked at this either. This was not the aim of this study.

Professor Taylor: Were you surprised to find no change in neuropsychological test results from preop to 5 days postoperatively?

Dr Wimmer-Greinecker: Actually we were not surprised, no. We have done other studies not only on heparin-coated systems, we also did studies on surface modification, and we also have done some studies on different oxygenators. In all those cases, we didn’t find any significances between groups evaluating $\text{S}100$ beta protein or our testing, so we actually were not surprised about those findings.

Professor Taylor: So your experience with cognitive testing is that you haven’t been able to pick up any change in any patient you studied undergoing open heart surgery?

Dr Wimmer-Greinecker: Among this study, there was one patient who had a transient neurologic damage. It was a paralysis of his left arm for 6 days. In this patient, we did have a correlation concerning the tests. This patient also showed an increase as well in $\text{S}100$ beta and in neuron-specific analysis.