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Effects of Phospholipid-Coated Extracorporeal Circuits on Clinical Outcome Parameters and Systemic Inflammatory Response in Coronary Artery Bypass Graft Patients

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ABSTRACT

Introduction: The use of extracorporeal circulation (ECC) during coronary artery bypass graft (CABG) surgery is associated with a systemic inflammatory response due to the contact of blood with artificial surfaces. The clinical relevance of ECC-related systemic inflammation varies with the patient, and such inflammation may be accompanied by intermittent organ dysfunction and an increased catecholamine requirement. We investigated the effects of a new phospholipid coating system of ECC on systemic inflammatory response and clinical outcome following CABG.

Methods: Patients scheduled for CABG surgery were prospectively divided randomly into 2 patient groups: patients using noncoated ECC materials and patients using phospholipid-coated ECC materials. Clinical data measured perioperatively included hemodynamics, aortic clamp time, duration of bypass, time to extubation, catecholamine requirement, length of intensive care unit (ICU) stay, postoperative blood loss, and amount of blood transfused. In addition, blood samples were collected before cannulation and at 2, 24, and 48 hours postoperative. Cytokines (tumor necrosis factor α [TNF-α] and interleukin 10 [IL-10]) and P-selectin were measured with an enzyme-linked immunosorbent assay. Plasma nitrate/nitrite levels (NOx) were determined by the Griess reaction.

Results: A significant increase of TNF-α level was noted in the uncoated control group only. In the uncoated group, IL-10 levels significantly increased at 2 hours postoperative, whereas levels remained unchanged in the phospholipid coating group. P-selectin increased 2 hours postoperative in the uncoated group, and no significant changes were noted in the phospholipid coating group. At 24 hours postoperative, total plasma NOx production significantly increased in the phospholipid coating group but remained constant in the control group. No significant differences with respect to postoperative parameters (time to extubation, ICU stay, amount of bleeding, blood transfused, and catecholamine requirement) were observed.

Conclusions: Phospholipid coating significantly reduces the systemic increase in proinflammatory and anti-inflammatory cytokines and P-selectin. Despite the comparable clinical outcomes in this study, the observed significant reduction in systemic inflammatory parameter values suggests an improved biocompatibility of ECC materials when they are coated with phospholipids.

INTRODUCTION

Extracorporeal circulation (ECC) is known to be associated with an undesirable inflammatory response syndrome that is either material-dependent (exposure of blood to artificial surfaces) or material-independent (surgical trauma, ischemia reperfusion injury, hypothermia, release of endotoxin). The inflammatory cascade includes activation of the complement system, release of various cytokines, and leukocyte activation, along with the expression of adhesion molecules and the release of various proinflammatory substances and oxidants such as superoxide or peroxynitrite [Steinberg 1993, Cremer 1996, Wan 1997].

One approach to minimize ECC-related inflammatory response syndrome may be the modification of the surface properties of the materials used. Recent data suggest that coating ECC compounds with phosphorylcholine (phospholipid) may mimic the main lipid component of the outer surface of a natural cell membrane [Hayward 1986]. Biological membranes are mainly composed of phospholipids ordered in a bilayer. The characteristic feature of the bilayer is their functional and compositional lipid asymmetry, which is thought to stem from the requirement of biological membranes to have asymmetric protein distribution across the bilayer [Op den Kamp 1979]. The inner surface is mainly composed of negatively charged phospholipids such as phosphatidylserine, whereas the outer leaflet predominantly consists of the zwitterionic phosphorylcholine-containing lipids. Ordered in a close-packed, planar array, phospholipids exhibit only a low level of interaction with proteins and cells [Scherphof 1983].

The phospholipid coating of cardiopulmonary bypass (CPB) circuits may provide improved biocompatibility with

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respect to the inflammatory reaction usually evoked by the exposure of blood to artificial surfaces.

We investigated in a prospective randomized study the potential benefits of phospholipid-coated surfaces with respect to clinical outcome parameters in patients scheduled for elective coronary artery bypass graft (CABG) surgery. In addition, we determined the production/release of inflammatory substances, such as tumor necrosis factor α (TNF-α) and P-selectin, as well as the production of nitric oxide and the anti-inflammatory cytokine, interleukin 10 (IL-10).

METHODS

Patient Selection and Characteristics of Extracorporeal Circuits

Thirty-six patients with coronary artery disease of various degrees undergoing CABG surgery using ECC were prospectively enrolled in this study. Consecutive patient selection was performed between August 2000 and April 2001. The study was approved by the Ethics Committee of the University of Munich, and all patients enrolled in the study gave written informed consent.

Patients were randomly divided into 2 groups. Group 1 (n = 18) underwent cardiopulmonary bypass using extracorporeal circuits coated with phosphorylcholine (Stöckert Physio, Munich, Germany). Coated components included arterial and venous lines, the cardioplegia line, cannulas, filters, and the membrane oxygenators. The identical circuit was used in group 2 (control; n = 18) without the use of coatings.

Exclusion criteria were impaired renal function with serum creatinine levels >1.6 mg/dL, liver function test results indicating impaired liver function, a history of diabetes mellitus, acute infections, and preexisting chronic inflammatory disease.

The extracorporeal circuit in both groups consisted of a membrane oxygenator, an arterial filter, a cardiotomy reservoir, and a pack of custom polyvinyl chloride tubing. Priming volume was 1100 to 1200 mL of crystalloid solution. Systemic heparinization was performed by applying 400 IU/kg body weight prior to cannulation, and the activated clotting time was maintained above 400 seconds during CPB. Six hundred to 700 mL of cardioplegic solution (Custodiol, Dr. Franz Köhler Chemie, Alsbach-Hahnlein, Germany) was administered via the aortic root. Surgery was performed with moderate hypothermia (34°C). Management of general anesthesia was identical in both patient groups.

All staff members involved in the perioperative care of study patients were blinded with regard to the ECC devices used.

Peripheral venous blood samples for the determinations of TNF-α, IL-10, P- selectin, and nitric oxide levels were collected 30 minutes prior to surgery, as well as at 2, 24, and 48 hours postoperative. Samples were centrifuged immediately, and the plasma samples were stored in liquid nitrogen for further processing.

Measurement of Cytokines and P-Selectin

Measurements of TNF-α and IL-10 levels were performed by means of commercially available enzyme-linked immunosorbent assays (R&D Systems, Wiesbaden, Germany). The appropriate volume of sample or standard was applied to a 96-well microtiter plate precoated with the corresponding monoclonal antibody. After the aspiration of the wells, plates were washed with a specific surfactant provided by the manufacturer. A solution of enzyme-linked polyclonal antibody and substrate was added to each well. The optical density of each well was read at the appropriate wavelength.

Measurement of Plasma Nitrate/Nitrite Levels

The Griess reaction was used for the determination of plasma nitrate/nitrite (NOx) levels. After deproteinization by ultrafiltration, the samples were incubated for 30 minutes at 37°C. Plasma nitrate was reduced to nitrite by adding 0.1 U/mL nitrate reductase in 2.5 mM phosphate buffer containing 5.0 μM flavin adenine dinucleotide and 50 μM reduced nicotinamide adenine dinucleotide phosphate. The final sample volume was 106.7 μL. Oxidation was carried out by applying 6.7 μL of a reaction solution consisting of 60 μL lactate dehydrogenase diluted 1:10 and 140 μL pyruvate. The reaction was subsequently incubated for 5 minutes at 37°C. The Griess reagent was then added, followed by a final incubation of 10 minutes at 37°C.

Spectrophotometric analysis was done at 540 nm, and the NOx content was calculated from the standard curve performed with each experiment.

Statistical Analysis

All data are presented as the mean ± SD. Dichotomous variables were analyzed with the Fisher exact test. A P value of <.05 was considered statistically significant. Analysis of biochemical parameter values over time was performed by analysis of variance followed by the Bonferroni post hoc analysis. Results were considered significant for $P < .05$.

RESULTS

Clinical Outcome Parameters

Eighty-four percent of the patients in both groups were men. There were no significant differences between the groups in age distribution, male-female ratio, preoperative ejection fraction, risk factors for coronary artery disease, number of peripheral anastomoses, CPB time, and cross-clamp time (Table 1).

In addition, no significant differences occurred with respect to postoperative clinical parameters, including blood loss, time to extubation, length of stay in the intensive care unit (ICU), and postoperative requirement for inotropes (Table 2).

TNF-α Plasma Levels

There were no significant differences in the preoperative levels of TNF-α between the 2 groups (Figure 1A). A significant increase in TNF-α levels was observed at 2 hours postoperative in the uncoated control group, whereas the levels in the phosphorylcholine coating group remained unchanged at all times of determination. Plasma levels of TNF-α did not return to baseline within 48 hours of observation (Figure 1A).

Table 1. Patient Characteristics*

Parameter	Control (n = 18)	Phosphorylcholine (n = 18)	P
Age, y	60 ± 11	67 ± 10	NS
Sex, % male	83	85	NS
Height, cm	172 ± 9	168 ± 9	NS
Weight, kg	82 ± 11	77 ± 10	NS
Body surface area, m^2	1.9 ± 0.16	1.8 ± 0.16	NS
Ejection fraction (preoperative), %	61 ± 11	63 ± 12	NS
Creatinine, mg/dL	1.1 ± 0.36	1.2 ± 0.36	NS
CAD class	2.17 ± 0.72	2.71 ± 0.59	NS
No. of risk factors	3.2 ± 1.1	3.3 ± 1.3	NS
Bypass time, min	68 ± 18	86 ± 27	NS
Aortic cross-clamp time, min	48 ± 12	58 ± 20	NS
No. of bypasses			
Veins	1.8 ± 1	2.2 ± 0.9	NS
Arteries	1.2 ± 0.3	1.1 ± 0.9	NS

*Measurements are presented as the mean ± SD. NS indicates not significant; CAD, coronary artery disease.

IL-10 Plasma Levels

Preoperatively measured plasma levels of IL-10 in the 2 groups were comparable. A significant increase in IL-10 plasma levels was noted within the groups at 2 hours postoperative. At this time, plasma IL-10 levels were significantly higher in the uncoated control group. In both groups, levels decreased to preoperative baseline levels at 24 hours postoperative (Figure 1B).

Plasma P-Selectin Levels

Plasma levels of P-selectin did not differ preoperatively between the 2 groups. At two hours postoperative, a significant elevation in P-selectin levels was observed in the uncoated control group, and these levels decreased to baseline again at 24 hours postoperative. In the phospholipid coating group, P-selectin levels did not show any significant change during the observation period (Figure 2).

Table 2. Postoperative Clinical Parameters*

Parameter	Control (n = 18)	Phosphorylcholine (n = 18)	P
Leukocyte count, × 10^9/L	9 ± 2	10 ± 3	NS
Platelet count, × 10^9/L	152 ± 32	147 ± 57	NS
Hematocrit, %	27	27	NS
CT drainage, mL/48 h	863 ± 614	873 ± 346	NS
Blood given, mL	108 ± 206	0	NS
FFP given, mL	33 ± 115	0	NS
Time to extubation, h	10.9 ± 5.4	11.8 ± 4.6	NS
Length of ICU stay, d	1.75 ± 0.62	1.45 ± 0.54	NS
Inotropes			
Epinephrine, mg/24 h	0.05 ± 0.1	0.46 ± 1.5	NS
Norepinephrine, mg/24 h	5.32 ± 5.9	2.77 ± 4.06	NS

*Measurements are presented as the mean ± SD. NS indicates not significant; CT, chest tube; FFP, fresh frozen plasma; ICU, intensive care unit.

Plasma NOx Levels

Baseline preoperative total NOx levels were comparable in the 2 groups. At 24 hours postoperative, total NOx increased significantly in the phospholipid coating group and came

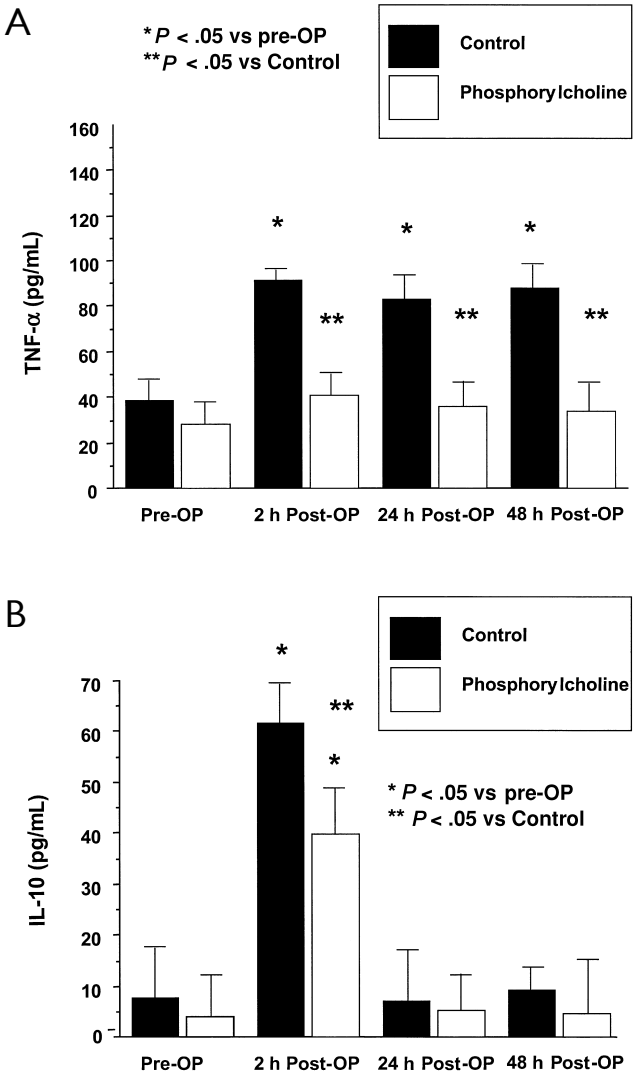

Figure 1. No significant differences in levels of preoperative tumor necrosis factor α (TNF-α) occurred between groups (A). A significant increase was observed at 2 hours postoperative in the uncoated control group, and levels in the phosphorylcholine coating group remained constant at all times of determination. Plasma levels of TNF-α did not decrease within 48 hours of observation in the control group. Preoperatively measured levels of interleukin 10 (IL-10) in plasma were comparable in both groups (B). Significant increases in IL-10 plasma levels occurred at 2 hours postoperative within both groups. Plasma IL-10 levels were significantly higher at this time in the uncoated control group and decreased to preoperative baseline levels at 24 hours postoperative in both groups. OP indicates operative.

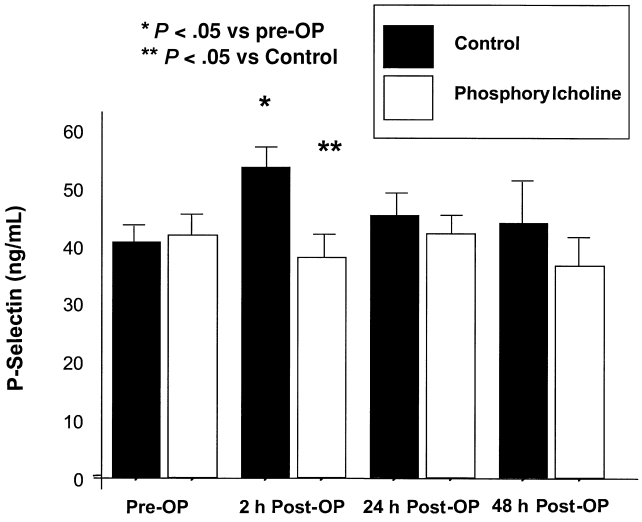

Figure 2. Plasma levels of P-selectin did not differ between the 2 groups preoperatively. At 2 hours postoperative, P-selectin levels were significantly elevated in the uncoated control group and came down to baseline again only at 24 hours postoperative. In the phosphorylcholine coating group, P-selectin levels showed no significant changes at all times of determination.

down to baseline levels within the following 24 hours, whereas levels in the control group remained unchanged (Figure 3).

Plasma Creatine Kinase Levels

As shown in Figure 4, no significant differences were noted with regard to preoperative and postoperative levels of creatine kinase. Electrocardiography and echocardiography results verified that the comparable postoperative increases in creatine kinase levels in both groups were not related to any acute ischemic events.

DISCUSSION

Recent evidence suggests that coating the extracorporeal circuits with phospholipids may provide protection against blood activation by contact with the materials' surfaces [De Somer 2000]. The same group reported beneficial results regarding complement activation in dogs in vivo [De Somer 1999].

The present study was performed to compare the clinical and biochemical effects of phospholipid-coated ECC circuits with the effects of uncoated circuits in patients undergoing CABG surgery.

Our results clearly demonstrate for the first time that coating ECC circuits with phospholipids significantly reduces the systemic inflammatory and compensatory anti-inflammatory response following the use of ECC. No differences are observed at the myocardial level, as reflected in the comparable release of creatine kinase and in the similar clinical outcome parameter values for both groups.

However, our findings may be of importance because systemic inflammation following the use of ECC is associated with increased patient morbidity [Wan, 1999]. Enhancement of the biocompatibility of artificial surfaces may help to reduce the inflammation-related organ dysfunction frequently observed in patients undergoing open heart surgery using ECC.

The pathophysiologic consequences may not be obvious from the clinical outcome parameters assessed. First, patient selection excluded severely diseased patients with multiple morbidities who are known to be at higher risk for postoperative complications and prolonged recovery periods. Second,

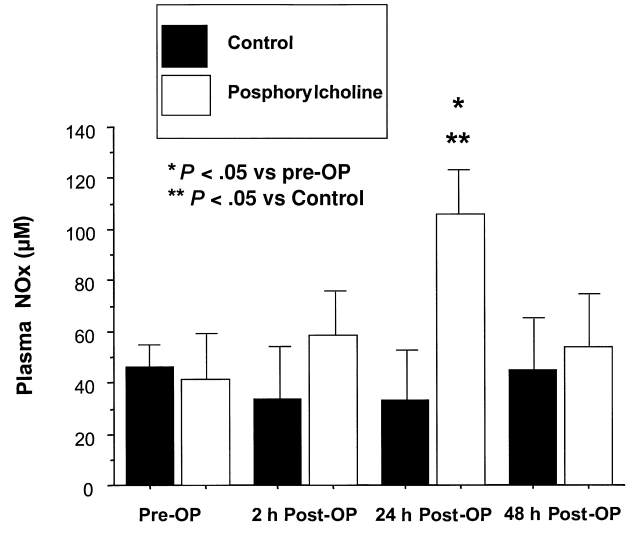

Figure 3. Baseline preoperative total nitrate/nitrite (NOx) levels were comparable in the 2 groups. Total NOx level increased significantly in the phosphorylcholine coating group at 24 hours postoperative and came down to baseline levels in the following 24 hours; control group levels remained unchanged.

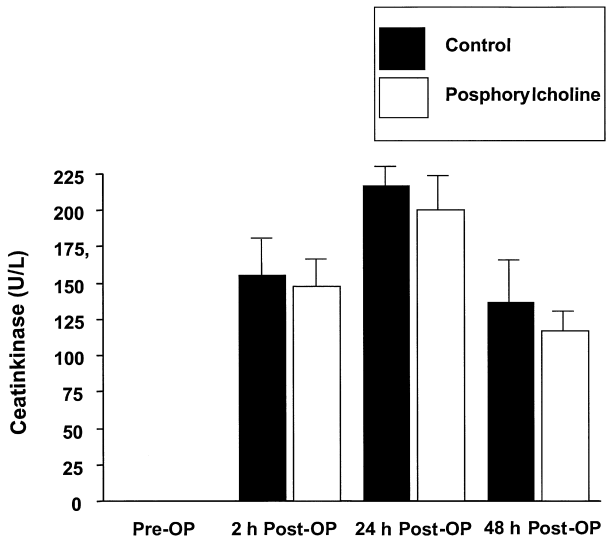

Figure 4. Preoperative and postoperative levels of creatine kinase showed no significant differences. The comparable increases in postoperative creatine kinase levels in the 2 groups were unrelated to any acute ischemic events.

the evaluations of clinical outcome parameters such as length of ICU stay and time to extubation are difficult because they may depend on the experience and expectations of the staff. Third, the duration of CPB averaged approximately 1 hour, and the phospholipid coating may become important in organ protection and clinical outcome when the ECC period is prolonged.

A new finding in the present study is the significant reduction of TNF-α levels in the phospholipid coating group (Figure 1A). Increased levels of TNF-α during and after the use of ECC have been shown to contribute to myocardial dysfunction and hemodynamic instability [Czerny 2000]. TNF-α has been shown to play an important role in heart failure by causing left ventricular dysfunction, precipitating pulmonary edema, reducing peripheral organ perfusion, and inducing ventricular remodeling [Kapadia 1999]. One mechanism may be a TNF-α–mediated induction of nitric oxide synthase and the resultant promotion of negative inotropic effects on the heart [Birks 1997]. In addition, TNF-α directly regulates the expression/activation of several other mediators, such as adhesion molecules (P-selectin and ICAM-1, among others), and exerts direct cytotoxic effects on tissues via specific TNF receptors [Ferrari 1995].

Adhesion molecules are important mediators and markers for cellular activation. They mediate adhesion of platelets and neutrophils to the endothelium. P-selectin is responsible for the initial tethering and rolling of leukocytes to the endothelium, and systemic levels of P-selectin have been shown to increase in patients undergoing CPB [Wildhirt 2001]. Reduction of soluble P-selectin levels under inflammatory conditions may reduce the interaction of platelets and leukocytes with the endothelium and result in decreased thrombogenicity and improved microcirculatory blood flow regulation [Lorant 1993]. In fact, increased levels of soluble P-selectin have recently been shown to be associated with a risk for future cardiovascular events [Ridker 2001].

In the present study, we observed a significant elevation of plasma NOx levels at 24 hours postoperative in the phospholipid coating group, whereas no changes were noted in the noncoated ECC group of patients. The cellular source of the NOx and the potential significance of these findings remain speculative. There is evidence from previous studies suggesting that ECC down-regulates endothelial nitric oxide synthase and that this event possibly contributes to endothelial dysfunction, enhanced thrombogenicity, and impaired blood flow regulation. Moreover, NOx bioavailability may be reduced under inflammatory conditions because of the presence of various oxidants such as superoxide. Higher NOx levels occurring early after the use of ECC in the phospholipid coating group and corresponding with a reduced inflammatory response may reflect a better preservation of NOx production or availability. However, this hypothesis cannot be proven by the present study.

The anti-inflammatory cytokine IL-10 is supposed to play a regulatory role by controlling the inflammatory response to injury [Fernando 2000]. It may be up-regulated during CPB to compensate for the action of proinflammatory cytokines [Diegeler 2000]. Indeed, elevated levels of IL-10 have been shown to be associated with myocardial dysfunction in low-risk coronary artery bypass surgery [Wei 2001]. The importance of compensatory up-regulation of IL-10 for myocardial protection has been shown by Jones and colleagues, who demonstrated that an IL-10 deficiency is associated with an enhanced myocardial infiltration of neutrophils following ischemia/reperfusion [Jones 2001]. In addition, IL-10 transfection has been shown to prolong graft survival in a rat heart allograft model, suggesting a potent inhibitory effect of this cytokine on T-cell function [Zuo 2001]. The significantly lower counter-regulatory up-regulation, as shown in patients receiving phospholipid-coated ECC, may be indicative of an overall reduction in the systemic inflammatory response in this group.

There is evidence from previous work suggesting that ECC per se accounts for a significant level of damage to myocardial cells [Czerny 2000]. Off-pump CABG surgery has been shown to significantly reduce the release of troponin and creatine kinase, compared with patients undergoing CABG with the use of ECC [Wildhirt 2000, Penttila 2001]; however, no significant differences were noted in the present study between groups with regard to myocardial protection, as indicated by the comparable releases of creatine kinase and the similar dosages of inotropes given to the 2 groups.

Taken together, our results show no obvious benefit of phospholipid coating with regard to clinical outcome parameter values in this selected group of patients. However, the phospholipid coating significantly reduces the inflammatory and compensatory anti-inflammatory response to CPB. The results imply a potential advantage of phospholipid coating in cases of extended CPB, in cases involving deep hypothermia, or in patients with advanced comorbidity. This issue needs to be addressed in further prospective randomized trials.

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