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Clinical experience with a novel endotoxin adsorbtion device in patients undergoing cardiac surgery

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Endotoxaemia is thought to occur in cardiac surgery using extracorporeal circulation (ECC) and a positive correlation has been proposed between the magnitude of endotoxaemia and risk for postoperative complications. We studied the effects of a new endotoxin adsorber device (Alteco® LPS adsorber) in patients undergoing cardiac surgery with ECC, with special reference to safety and ease of use. Fifteen patients undergoing coronary artery bypass and/or valvular surgery were studied. In 9 patients, the LPS Adsorber was included in the bypass circuit between the arterial filter and the venous reservoir. Flow through the adsorber was started when the aorta was clamped and stopped at the end of perfusion. Flow rate was kept at 150 ml/min. Six patients served as controls with no adsorber in the circuit. Samples were taken for analysis of endotoxin, TNFα, IL-1ß and IL-6 as well as complement factors C3, C4 and C1q. Whole blood coagulation status was evaluated using thromboelastograpy (TEG) and platelet count. No adverse events were encountered when the adsorber was used in the circuit. Blood flow through the device was easily monitored and kept at the desired level. Platelet count decreased in both groups during surgery. TEG data revealed a decrease in whole blood clot strength in the control group while it was preserved in the adsorber group. Endotoxin was detected in only 2 patients and IL-1ß in 4 patients. IL-6 decreased in both groups whereas no change in TNF concentrations was found. C3 fell in both groups, but no changes wer found in C4 and C1q. The Alteco® LPS adsorber can be used safely and is easy to handle in the bypass circuit. No complications related to the use of the adsorber were noted. The intended effects of the adsorber, i.e. removal of endotoxin from the blood stream could not be evaluated in this study, presumably due to the small number of patients and the relatively short perfusion times. *Perfusion* (2009) **24**, 13–17.

Key words: adsorbtion; cardiopulmonary bypass; endotoxin; inflammatory mediators

Introduction

Extracorporeal circulation (ECC) induces an inflammatory response in the patient.[1,2] The pathogenesis of this process is multifactorial and includes the effects of contact between blood and foreign surfaces in the bypass circuit, as well as possible immunological responses to drugs given during the procedure, e.g. protamine. Endotoxaemia during ECC is a well described phenomenon, generally considered to be due to translocation of gram-negative bacteria from the intestinal tract.[3] The cause and magnitude of this translocation is probably a combination of relative hypoperfusion in the intestinal circulation during bypass and previous vascular disease manifested in this region. There is a well-established relationship between the magnitude of the activation of the overall inflammatory response and adverse clinical outcome after cardiac surgery using ECC.[3,4] Various techniques have been used to reduce the inflammatory response,[5–7] including changes in the bypass circuit and pharmacological manipulation of the inflammatory process. The clinical effects of these techniques have, so far, been rather discouraging. In this study, we describe a method specifically aimed at reducing the possible endotoxin load caused by ECC. Evaluation of the safety of the method and possible effects on complement and cytokine systems, as well as effects on platelet count and function, were studied.

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Materials and methods

The Alteco LPS® Adsorber (Alteco Medical, Lund, Sweden) consists of porous polyethylene discs to which is bound a specific polypeptide that binds to the A-moiety of lipopolysacharide (LPS). The discs are enclosed in a polycarbonate hub with connections

for blood supply. The priming volume of the device is 80 ml and the total surface area of the discs is 3 m^2. In vitro tests have shown that the adsorber reduces LPS levels from 1 EU/ml in 5000 ml bovine blood to less than 0.05 EU/ml during recirculation for 60 min at a flow rate of 100 ml/min (information supplied by the manufacturer).

Fifteen patients scheduled for elective coronary and/or valvular surgery were included in the study. The study protocol was approved by the ethical committee of the hospital and written consent was obtained from all patients. Exclusion criteria were ongoing myocardial infarction, unstable circulation, ejection fraction (EF) less than 35% and known liver and/or coagulation disorder.

Nine patients had the LPS Adsorber included in the ECC circuit, with afferent flow taken from the arterial filter and efferent flow going into the venous reservoir (Figure 1). Six patients served as controls with no adsober in the circuit.

Anaesthesia was induced with an infusion of propofol (Propofol Lipuro®, Braun Medical) and fentanyl (Leptanal®, Janssen-Cilag) and vecuronium (Norcuron®, Organon) was used for muscle relaxation. Mechanical ventilation was established and maintenance of anaesthesia was achieved with repeated doses of fentanyl and continuous infusion of propofol (3 mg/kg/h) during ECC. The patients were connected to the heart-lung machine (SIII, Sorin Group München, Munich, Germany) via standard cannulation in the aortic root and the right atrium. A hollow-fibre oxygenator with a venous reservoir and built-in heat exchanger (Compactflow EVO®, Sorin Group, Modena, Italy) and a 40µm arterial filter (D734 MICRO 40 A®, Sorin Group, Modena, Italy) were used in the ECC circuit. Blood flow in the ECC was calculated at 2,4l/min/m^2. Heparin, at an initial dose of 400 U/kg, was given before cannulation; additional doses were given, if necessary, to maintain an activated clotting time (ACT) of 420 sec.

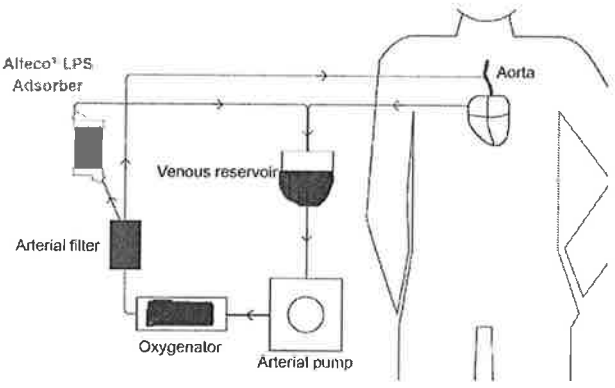

Figure 1 Incorporation of the LPS adsorber in the ECC circuit. Blood is taken from the arterial filter through the adsorber and into the venous reservoir.

Blood flow through the adsorber was initiated when the aorta was clamped and terminated at the time of weaning from the ECC. The blood flow velocity through the adsorber was continuously monitored by a CardioMed® Transonic Doppler flow velocity monitor (Vingmed AS, Oslo, Norway) and kept constant at 150 ml/h.

Blood samples for analysis of LPS, TNFα, Il-1ß, Il-6 and platelet count were taken before anaesthesia, 10 min after aortic clamping, at skin suture and 6 hours after skin suture. Platelets were also counted on the first postoperative day. Blood for LPS and cytokine analysis was immediately centrifuged and frozen pending further analysis. LPS was analysed using the Kinetic chromogene LAL test (Charles River Inc, Boston, MA, USA), Il-1 was analysed with an immunochemical method and for TNF and Il-6 a chemiluminiscense (Immulite® Siemens Healthcare Diagnostics, Deerfield, IL, USA) method was used. Samples for analysis of complement factors (C1q, C3 and C4) were taken before anaesthesia and 6 hrs after skin suture and analysed using a turbidometric method (Cobas Mira®, Roche Diagnostics Ltd, Rotkreutz, Switzerland). Whole blood coagulation profile was evaluated using thromboelastograpy (TEG®, Hemoscope Corp., Niles, IL, USA); samples were obtained before anaesthesia and after full reversal of heparin with protamine.

Statistics

Demographic and perfusion-related data are presented as median and range; all other data are presented as mean ±SEM. Correction for haemodilution was performed for the cytokine and complement data by using the following formula: corrected value=measured value x 40/measured haematocrit. Friedman repeated measurement on ranks was used for analysis of changes within the groups, and Mann Whitney's rank sum test was used for analysing differences between the groups. A *P*-value of less than 0.05 was considered significant.

Results

Demographic data are presented in Table 1. There were no differences between the groups regarding perfusion time or time for aortic clamping. The incorporation of the adsorber into the ECC circuit

Table 1 Demographic and technical data

	Age (years)	Perfusion time (min)	Clamping time (min)	Adsorption time (min)
Adsorber	66 (35–80)	104 (46–198)	71 (30–139)	85 (41–160)
Controls	73 (59–86)	118 (50–239)	83 (24–167)	

was uneventful in all patients and the blood flow through the adsorber was easily maintained at 150 ml/min. There was a significant difference in pre-anaesthetic platelet count, with higher values in the adsorber group ($P = 0.042$). Platelet count decreased in both groups during surgery, with no difference between the groups (Figure 2). The effects of ECC on the thromboelastic properties measured by TEG were surprisingly moderate. No differences were found in either the r-value (time to form the fibrin clot) or the k-value and angle (both considered to reflect the speed of clot formation). However, maximum amplitude (MA), reflecting the strength of the final clot, decreased significantly in the control group ($P > 0.001$), whereas no such change was seen in the adsorber group (Table 2).

Endotoxin was found in only 2 patients, one in each group, and both at skin suture. These two patients had long clamp times, 113 and 123 min. The results of the analysis of TNFα are shown in Table 3. No significant changes were noted in either group. IL-1ß was found in only 4 patients in the adsorber group and 3 patients in the control group (Table 3). Concentrations of IL-6 were significantly higher in the adsorber group before anaesthesia; a further increase was observed in both groups during the observation time with no difference between the groups. There was a significant decrease in C3 concentrations in both groups, whereas no changes were seen concerning C4 and C1q (Table 4).

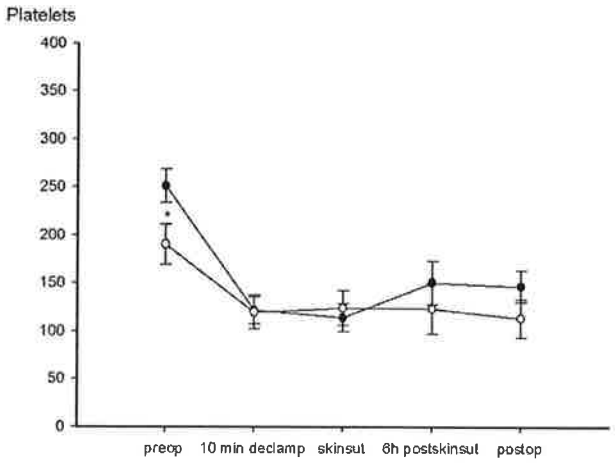

Figure 2 Platelet number in adsorber group (filled circles) and controls (open circles). Mean ± SEM. The decrease in platelet number is significant ($P < 0.05$, Anova on ranks) in both groups.

Discussion

Although the pathogenesis behind septic shock is multifactorial and involves activation of multiple cascade systems, LPS is considered to be a major contributor to the development of septic shock and its consequences in gram-negative sepsis.[7] Different techniques to decrease the total load of LPS have, thus, been evaluated.[8–10] Endotoxaemia is known to occur in cardiac surgery using ECC and a relationship between the magnitude of endotoxaemia and postoperative complications has been shown.[11] It would, therefore, be of interest to investigate methods aiming at reducing the endotoxic load in procedures such as those involving long perfusion, circulatory arrest and transplantations. In a recent animal study using ECC, the addition of a polymyxin-based LPS adsorber was reported to have positive effects concerning cytokine production and haemodynamic outcome. However, no data regarding the occurrence of LPS were shown.[12] Our primary goal was to evaluate the safety and usability of the LPS adsorber in patients undergoing surgery

Table 2 TEG-data

	Pre-anaesth	6 h post-op	t-test
R (min)			
Adsorber	6.1 ± 0.9	5.9 ± 0.8	ns
Controls	6.0 ± 0.7	7.5 ± 1.7	ns
k (min)			
Adsorber	1.8 ± 0.3	2.0 ± 0.4	ns
Controls	1.7 ± 0.3	3.3 ± 1.3	ns
MA (mm)			
Adsorber	67.0 ± 1.9	61.8 ± 2.8	ns
Controls	65.8 ± 2.3	48.6 ± 5.4	$P < 0.05$
Angle (centigrade)			
Adsorber	66.6 ± 2.6	59.9 ± 5.3	ns
Controls	66.2 ± 3,7	62.3 ± 1.2	ns

Samples taken before anaesthesia and after full reversal with protamin. R-value (min) represents the time to form fibrin clot. Angle and K (centigrade, min) represents the speed of clot formation. MA (mm) represents maximum clot strength. Mean ± SEM.

Table 3 Cytokine concentrations

	Pre-anaesth	10 min declamp	Skin suture	6 h post-op	Anova
TNF-α (pg/ml)					
Adsorber	7.18 ± 4.3	11.5 ± 4.4	11.9 ± 4.7	5.6 ± 1.9	ns
Controls	11.88 ± 10.1	4.0 ± 0.5	5.4 ± 0.9	5.4 ± 1.2	ns
IL-6 (pg/ml)					
Adsorber	9.60 ± 3.1	50.8 ± 21.6	42.2 ± 17.8	87.8 ± 21.5	$P < 0.05$
Controls	2.47 ± 0.6	38.7 ± 20.0	51.4 ± 25.3	117.6 ± 36.9	$P < 0.05$
IL1-β (pg/ml)					
Adsorber	0.12 ± 0.1	0.69 ± 0.40	0.93 ± 0.60	0.22 ± 0.2	ns
Controls	0.11 ± 0.07	0.01 ± 0.01	0.07 ± 0.07	0.01 ± 0.01	ns

TNF-α and Il-6 were found in all patients whereas Il-1β was only found in 4 patients in the adsorber group and 3 in the control group. Mean ± SEM.

with cardiopulmonary bypass and evaluate possible side effects of the treatment. Since all filter devices, including oxygenators, have a tendency to cause platelet trapping, resulting in a decrease in total platelet count and function, these factors were of special interest in this study. The efficacy of the Alteco® LPS Adsorber in reducing LPS concentrations has, so far, not been shown in clinical studies, although one case report has been published indicating positive effects of the device.[13] We did not encounter any technical or other problems when introducing the adsorber in the circuit. As seen in Figure 1, the efferent flow from the adsorber is connected to the venous reservoir, thus, avoiding any danger from possible air leakage. The inclusion of the adsorber resulted in a shunt flow of 150 ml/h; this effect is negligible in adult patients. However, it must be taken in to consideration should the device be used in paediatric patients. Monitoring and regulating the flow rate through the adsorber was uneventful and easily performed. The decrease in platelet count during surgery was expected and no difference between the groups was found. The results of the thromboelastography should be interpreted with caution. However, it is well worth noticing that the MA value was unchanged in the treatment group while it decreased in the control group, thus, indicating a preserved clot strength in the treatment group. A secondary goal of this study was to investigate if the inclusion of the adsorber in the ECC circuit had any effects on the inflammatory response and possible endotoxaemia. The fact that we were able to detect endotoxin in only 2 patients could indicate that endotoxaemia is not a common feature in procedures with relatively short perfusion and clamping times. However, it should be emphasized that analysis of LPS in human blood is notoriously difficult, mainly due to protein contamination.[14] Furthermore, choosing different sampling points might have yielded other results. The fact that Il-1ß was found in less than half of the patients in both groups supports the suggestion that the LPS load in these patients was fairly low. Our results concerning cytokines and complement factors should likewise be interpreted with caution. Other initiating events apart from LPS, such as foreign body contact, must be considered.

Table 4 Concentrations of complement factors

	Pre-anaesth	6 h post-op	t-test
C3 (g/l)			
Adsorber	1.44 ± 0.07	1.13 ± 0.17	$P < 0.05$
Controls	1.16 ± 0.05	0.97 ± 0.16	$P < 0.05$
C4 (g/l)			
Adsorber	0.33 ± 0.03	0.27 ± 0.05	ns
Controls	0.30 ± 0.03	0.25 ± 0.02	ns
C1q (%)			
Adsorber	113.9 ± 8.4	94.1 ± 8.1	ns
Controls	94.8 ± 5.0	83.1 ± 9.2	ns

Mean ± SEM.

In conclusion, we consider it safe to include the Alteco® LPS Adsorber in the ECC circuit. We did not encounter any adverse events neither did we detect any effects on coagulation status. The purpose of including the adsorber would be to decrease a possible LPS load caused by the extracorporeal circulation. In our study, with a small number of patients and relatively short perfusion times, no such increase in LPS in the systemic circulation was detected. Thus, further studies including patients in need of advanced surgery where long perfusion times and/or circulatory arrest could be anticipated are needed to evaluate potentially advantageous effects of the adsorber.

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