A clinical study on the effects of pulsatile cardiopulmonary bypass on the blood endotoxin levels

Levels of endogenous endotoxins have been reported to increase after cardiopulmonary bypass. Endotoxin levels have also been implicated in multiple organ failure and may contribute to the immunocompromised state seen after bypass. We evaluated the effects of pulsatile cardiopulmonary bypass circulation on endogenous endotoxin levels. The study population consisted of 15 consecutive adult patients who underwent cardiac operations with cardiopulmonary bypass. Pulsatile flow was used during aortic crossclamping in eight patients (group I) and nonpulsatile flow was used in the remaining seven patients (group II). Changes in blood endotoxin levels were monitored during aortic crossclamping, after release of the clamp, and after weaning from bypass. The blood endotoxin level at each stage was expressed as a percentage of the level at the beginning of bypass. Group I patients had a significantly lower blood endotoxin percentage than group II (from 20 to 120 minutes after the initiation of aortic crossclamping). In group I, the blood endotoxin percentage was nearly constant during aortic crossclamping. After release of aortic crossclamping, group I also had a lower blood endotoxin percentage than group II. Endogenous endotoxin levels appear to increase in the presence of intestinal congestion and ischemia. Improvement in intestinal circulation by pulsatile cardiopulmonary bypass may prevent increases in endogenous endotoxin levels by reducing these factors. (J THORAC CARDIOVASC SURG 1994;108:620-5)

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The term *endotoxin* was coined in 1892 by Pfeiffer after toxins found in a heat-resistant strain of cholera bacilli. In the 1950s, Westphal demonstrated that endotoxins were primarily lipopolysaccharides. Clinically, endotoxins were first measured by Levin, Tomasulo, and Oser¹ in 1968. They described a method of detecting endotoxins based on the aggregation and gel formation of minute amounts of endotoxins by *Limulus* lysate (a component of *Limulus* blood cells). Various methods have since been developed to analyze endotoxins qualitatively and quantitatively. By means of these methods, the diverse biolog-

ic activities of endotoxins have been discovered. Numerous reports have indicated that endotoxemia is involved in postoperative multiple organ failure^{2,3} and that levels may increase during cardiopulmonary bypass (CPB).⁴ Inasmuch as immune function appears to decrease after CPB,⁵⁻⁷ we hypothesized that reduction of blood endotoxin levels may help prevent postoperative multiple organ failure and achieve better surgical results. We⁸⁻¹⁰ have previously reported clinical and experimental studies on the usefulness of pulsatile CPB. In this study, we hypothesized that pulsatile CPB might decrease endogenous endotoxemia by improving splanchnic blood flow, and we examined the association between pulsatile CPB on the blood endotoxin levels. The aim of this study was to examine the usefulness of pulsatile CPB for reducing the endogenous endotoxin levels in the blood.

Patients and methods

Our study population consisted of 15 consecutive adult patients who underwent cardiac operations with CPB, and informed consent was obtained from each patient. Patients were

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Table I. Demographic and operative data

Patient No.	Age (yr)	Sex	Diagnosis	Procedure	Total CPB time (min)	Aortic crossclamp time (min)	Minimum rectal temperature (°C)	Flow
1	56	M	AP	CABG	425	173	24.2	P
2	52	M	AP	CABG	158	50	28.0	P
3	47	F	MS	MVR	247	130	20.7	P
4	61	F	MS	MVR	218	98	21.9	P
5	29	F	inECD	Correction	198	77	22.7	P
6	56	M	AP	CABG	296	175	24.7	P
7	62	F	ASD,MR	ASD closure, MVR	290	131	25.7	P
8	41	F	MS	MVR	195	104	24.7	P
9	68	M	AP	CABG	298	155	24.7	NP
10	65	M	AP	CABG	328	156	24.9	NP
11	57	M	AP	CABG	278	108	25.5	NP
12	61	M	AP	CABG	296	146	26.3	NP
13	60	M	AP	CABG	345	168	25.8	NP
14	63	F	MR	MVR	194	95	23.9	NP
15	29	F	inECD	Correction	152	93	25.5	NP

CPB, Cardiopulmonary bypass; AP, angina pectoris; MS, mitral stenosis; inECD, incomplete endocardial cushion defect; ASD, atrial septal defect; MR, mitral regurgitation; CABG, coronary artery bypass grafting; MVR, mitral valve replacement; P, pulsatile; NP, nonpulsatile.

enrolled between April 1988 and March 1989 (Table I). No study patient had any preoperative sign of clinical infection. The study was approved by the institutional review board and informed consent was obtained from each patient. There were eight men and seven women in the study. Patients' ages at the time of operation ranged from 29 to 68 years, with an average of 53.8 ± 12.1 years (mean $\pm$ standard deviation). The preoperative diagnosis was angina pectoris in nine patients, mitral stenosis in four patients, incomplete-type endocardial cushion defect in two patients, and a combination of atrial septal defect and mitral regurgitation in one patient. The surgical procedure was coronary artery bypass grafting in nine patients, mitral valve replacement in five, and total correction in three patients. Intraoperative anesthetic management was uniform for all patients. Pulsatile and nonpulsatile CPB flow was used during aortic crossclamping. Pulsatile flow was generated with a Sarns 7400 roller pump (Sarns Inc./3M Health Care, Ann Arbor, Mich.) at a pump flow rate of 2.4 L/min per square meter body surface area, a pulse pressure of 30 mm Hg, a mean blood pressure of 50 mm Hg, and a pulse rate of 70 beats/min. Pulsatile flow was used only during aortic crossclamping. Nonpulsatile flow was generated with a Sarns 7400 roller pump at a flow rate of 2.4 L/min per square meter body surface area and a mean blood pressure of 50 mm Hg. Oxygenation was provided by a membrane oxygenator in all cases (Capiiox E, Terumo Corp., Tokyo, Japan). A cold blood cardioplegic solution was administered intermittently via the aortic root and ice cold Ringer's solution was applied topically to the heart. The patients were divided into two groups on the basis of the use of pulsatile or nonpulsatile flow during CPB: the pulsatile flow group consisted of eight patients (group I) and the nonpulsatile flow group consisted of seven patients (group II). Blood endotoxin levels during aortic crossclamping, during rewarming after unclamping, and after weaning from the artificial heart-lung system were measured and expressed as a percentage of the level at the beginning of CPB (Et%).

Blood sampling for endotoxins. All blood sampling was performed in an aseptic fashion and endotoxin-free collection tubes were used. During CPB with the artificial heart-lung sys-

Table II. Patient data

	Group I (n = 8)	Group II (n = 7)
Age (yr)	50.5 $\pm$ 11.1	57.5 $\pm$ 13.0
Total CPB time (min)	253.3 $\pm$ 84.0	270.1 $\pm$ 70.9
Aortic crossclamp time (min)	117.3 $\pm$ 43.9	131.6 $\pm$ 31.8
Minimum rectal temperature (°C)	24.1 $\pm$ 2.3	25.2 $\pm$ 0.29

Values are expressed as mean $\pm$ standard deviation of the mean. There were no significant differences between the groups for these parameters.

tem, the blood was sampled from the venous route of the system. After the patient was weaned from CPB, the blood was sampled from the pulmonary artery with a Swan-Ganz catheter (Baxter Healthcare Corp., Edwards Div., Irvine, Calif.). Blood sampling was performed during the following intervals: (1) at the beginning of CPB, (2) every 20 minutes after initiation of aortic crossclamping, (3) every 30 minutes after release of the aortic crossclamp, (4) immediately after weaning from CPB, (5) 2 hours after weaning from CPB, and (6) 12 hours after weaning from CPB.

Measurement of endotoxins. Endotoxin levels were measured with an endotoxin-specific chromogenic test (Endospecy test; Seikagaku Kogyo, Tokyo, Japan). This test consists of factor G-free *Limulus amoebocyte* lysate and a chromogenic substrate.⁹ The sample was pretreated with perchloric acid and sodium hydroxide before endotoxin levels were determined. The endotoxin level at the beginning of CPB was designated as 100% (baseline level). Endotoxin levels on subsequent occasions were expressed as a percentage relative to the baseline level (Et%) after correction for hematocrit levels. This allowed for comparisons of endotoxin levels at a hematocrit value of 25%.

Statistical analysis. Values were expressed as mean $\pm$ standard deviation. The significance of differences was tested by

Table III. Changes in endotoxin levels during aortic crossclamping

Before clamping	Time after start of aortic crossclamping					
	20 min	40 min	60 min	80 min	100 min	120 min
Group I						
Range	24%-150%	37%-123%	26%-104%	56%-100%	86%-105%	47%-178%
100%	83% $\pm$ 39%	93% $\pm$ 33%	69% $\pm$ 33%	87% $\pm$ 18%	97% $\pm$ 8%	93% $\pm$ 61%
(n = 8)	(n = 8)	(n = 8)	(n = 7)	(n = 6)	(n = 5)	(n = 4)
Group II						
Range	65%-294%	100%-325%	38%-282%	137%-260%	130%-480%	117%-480%
100%	172% $\pm$ 84%*	183% $\pm$ 98%*	152% $\pm$ 93%*	185% $\pm$ 53%*	251% $\pm$ 146%*	245% $\pm$ 100%*
(n = 7)	(n = 7)	(n = 7)	(n = 7)	(n = 7)	(n = 5)	(n = 4)

Values are expressed as mean $\pm$ standard deviation of the mean.

* $p < 0.05$ when compared with group I.

Table IV. Changes in endotoxin levels after release of aortic crossclamp

Before clamping	30 min after release of ACC	60 min after release of ACC	Immediately after CPB	2 hr after CPB	12 hr after CPB
Group I					
Range	50%-150%	16%-354%	32%-107%	41%-234%	33%-295%
100%	88% $\pm$ 33%	74% $\pm$ 45%	57% $\pm$ 24%	102% $\pm$ 62%	172% $\pm$ 106%
(n = 8)	(n = 8)	(n = 8)	(n = 8)	(n = 8)	(n = 8)
Group II					
Range	68%-233%	65%-250%	54%-336%	35%-424%	63%-1283%
100%	171% $\pm$ 61%*	143% $\pm$ 68%*	143% $\pm$ 116%*	175% $\pm$ 158%*	362% $\pm$ 215%
(n = 7)	(n = 7)	(n = 7)	(n = 7)	(n = 7)	(n = 7)

Values are expressed as mean $\pm$ standard deviation of the mean. ACC, Aortic crossclamp; CPB, cardiopulmonary bypass.

* $p < 0.05$ when compared with group I.

means of a Mann-Whitney U test. Differences were considered to be significant at a p value of less than 0.05. Statview II (Abacus Concepts, Inc.) statistical software for the Macintosh computer was used for statistical analyses.

Results

The total CPB time did not differ significantly between group I (158 to 425 minutes, 253.3 ± 84.0 minutes) and group II (152 to 345 minutes, 270.1 ± 70.9 minutes). Aortic crossclamping time also did not differ significantly between group I (50 to 175 minutes, 117.3 ± 43.9 minutes) and group II (93 to 168 minutes, 131.6 ± 31.8 minutes). Finally, the minimum rectal temperature also did not differ significantly between group I (21.9° to 28.0° C, $24.1^\circ \pm 2.3^\circ$ C) and group II (23.9° to 26.3° C, $25.2^\circ \pm 0.3^\circ$ C) (Table II). One patient with mitral stenosis (case 4, group I) died on postoperative day 16 after mitral valve replacement because of multiple organ failure caused by a low cardiac output syndrome. The other 14 patients had no significant postoperative complications. Endotoxins were not found in any of the priming fluids (0 pg/ml). Additionally, after the priming fluid was mixed in the extracorporeal circuit and circulated for

10 minutes, endotoxins were still not found in any of our patients (0 pg/ml). No great variation was observed among the patients. The hematocrit value during CPB was 23% to 28%. No patients required any blood transfusions.

Changes in Et % during aortic crossclamping (Table III, Fig. 1). Twenty minutes after the initiation of aortic crossclamping, the Et% in group I ($83\% \pm 39\%$) was significantly lower than in group II ($172\% \pm 84\%$) ($p < 0.05$). This parameter then remained significantly higher in group II than in group I until 120 minutes after the initiation of aortic crossclamping ($p < 0.05$). In group II, the Et% increased slightly during the course of aortic crossclamping. In group I, however, the Et% did not change during the course of aortic crossclamping.

Changes in Et % after release of aortic cross clamp (Table IV, Fig. 2). At two points during the rewarming processes (30 and 60 minutes after release of the aortic clamp), the Et% was significantly lower in group I ($88 \pm 33\%$ at 30 minutes and $74\% \pm 45\%$ at 60 minutes) ($p < 0.05$) than in group II ($171\% \pm 61\%$ at 30 minutes and $143\% \pm 68\%$ at 60 minutes). In group II, the Et% at 60 minutes after release of the aortic crossclamp (that is,

Fig. 1. Changes in Et% during aortic crossclamping in the two groups. SD, Standard deviation. * $p < 0.05$ when compared with group I.

Fig. 2. Changes in Et% after release of aortic crossclamp in the two groups. * $p < 0.05$ when compared with group I. AR-ACC, After release of aortic crossclamp; SD, standard deviation.

the point when rewarming was nearly complete) was lower than the Et% recorded 30 minutes after release of the aortic crossclamp. This difference, however, was not statistically significant.

Changes in Et% after weaning from CPB (Table IV, Fig. 2). The Et% was significantly higher in group II than in group I immediately and 2 hours after weaning from CPB (group I, $57\% \pm 24\%$ and $102\% \pm 62\%$; group II, $143\% \pm 116\%$ and $175\% \pm 158\%$, respectively). The

Et% remained higher in group II ($362\% \pm 215\%$) than in group I ($172\% \pm 106\%$) even 12 hours after cessation of CPB.

Discussion

Endotoxins, that is, lipopolysaccharides, constitute the adventitia of the cell wall of gram-negative bacilli and have diverse biologic activities and may contribute to the development of multiple organ failure.^{12, 13} Elevated

blood levels of endotoxins (i.e., endotoxemia) have been noted in patients with multiple organ failure. An impairment of reticuloendothelial or immune function may lead to endogenous endotoxemia by intestinal gram-negative bacilli, such as *Escherichia coli*. It has been noted that immune function appears to be depressed in patients immediately after CPB⁵⁻⁷ and that the risk for multiple organ failure caused by endogenous endotoxemia is high during this period. Only a few reports examining endotoxin levels during CPB have been published, and no reports assessing endotoxin levels during pulsatile CPB have appeared. Rocke and associates⁴ have reported that blood endotoxin levels increase during aortic crossclamping. Jansen and colleagues¹⁴ have reported that the release of endotoxin, associated with the formation of tumor necrosis factor, plays an additional role in the development of the whole-body inflammatory reaction, independent of the material activation. Andersen and colleagues¹⁵ have also reported that patients "free of endotoxins" before the operation had rising concentrations to 64 ng/L by the end of CPB, with a peak median level of 95 ng/L at 90 minutes after CPB. Nilsson and coworkers have reported that endotoxin levels increase during CPB. They suggested that this endotoxin release may be from the extracorporeal equipment. In our study, however, endotoxins were not found in any of the priming fluids. The increase in endotoxin after release of the aortic crossclamp might be explained by the preceding hypoperfusion of the splanchnic bed by reduced peak arterial pressure associated with the nonpulsatile flow of the pump during CPB.⁴ In the present study, patients in the nonpulsatile CPB group had a significant increase in blood endotoxin levels with aortic crossclamping. In the pulsatile CPB group, however, no significant elevation was noted in endogenous endotoxin levels during aortic crossclamping. Thus our study demonstrates the clinical utility of pulsatile CPB in preventing increases in endogenous endotoxin levels.

Totsuka and associates,^{3,17} have reported three possible routes for the transfer of endogenous endotoxins into the blood: (1) transfer from the intestine into the portal vein; (2) transfer from the intestine into the systemic circulation via the intestinal lymphatics, thoracic duct, and venous angle; and (3) adsorption into the portal vein and intestinal lymphatics from the peritoneal cavity. An important relationship was noted between the lymphatic system and the intestine in endogenous endotoxemia. This relationship is based on observations that demonstrate that thoracic duct drainage significantly reduces blood endotoxin levels. Previous studies have shown that CPB can lead to ischemic damage of organs perfused by the splanchnic circulation.^{18,19} During CPB, the intestine

tends to become edematous and congested because of perfusion of blood with low osmotic pressure. Thus endogenous endotoxin levels in intestinal blood are likely to increase during CPB. The liver also tends to become congested during CPB, and Kupffer cell function is believed to be disturbed during this period,²⁰ leading to further elevations in hepatic blood endotoxin levels. A possible mechanism to explain the decrease in endotoxin levels noted with pulsatile CPB is that pulsatile CPB increases intestinal²¹ and hepatic blood flow²² and improves splanchnic circulation, thereby leading to a decrease in the transfer of endogenous endotoxins into the blood and an improvement in the endotoxin-disposing capability of the liver. Fortunately, in our study, endotoxin-induced multiple organ failure was not recognized. However, we consider that pulsatile CPB should be used during aortic crossclamping in cardiac operations for patients whose status is poor before the operation.

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