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Effects of Hypothermia on Blood Endogenous Endotoxin Levels During Cardiopulmonary Bypass

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KARDİYOPULMONER BYPASS'DA HİPOTERMİNİN KAN ENDOJEN ENDOTOKSİN DÜZEYLERİNE ETKİSİ

Endotoksinler, lökositleri ve kompleman sistemini aktive ederek, ateşten septik şoka kadar değişen bir kliniğe sebep olurlar. Kardiyopulmoner bypassta endojen endotoksemi olduğu bilinmekle beraber, hipoterminin buradaki rolü hakkında bir bilgi yoktur. Biz çalışmamızda orta (24-28°C) ve hafif (32-34°C) hipoterminin kan endojen endotoksin düzeylerine etkisini araştırdık. CABG olacak toplam 20 hasta iki gruba ayrılarak Grup 1'deki 10 hastaya, aortik kros klemp sırasında, orta derecede hipotermi, Grup 2'ye ise hafif hipotermi uygulandı. Grup 1'deki ortalama rektal ısı 26.8 ± 1.2°C olurken, Grup 2'de 33.8 ± 0.8°C oldu. Kan örnekleri KBP'dan önce, aortik kros klemp sırasında, kros klempten hemen sonra, kros klempten 20 dakika sonra, KBP'dan hemen sonra ve postoperatif 24. saatte alındı. KBP öncesi örneklerde her iki grupta da endotoksine rastlanmazken, diğer örneklerde kan endotoksin düzeyleri Grup 1'de, Grup 2'ye oranla anlamlı ölçüde yüksek bulundu. Muhtemel sebep, hipotermi derinleştikçe artan intestinal iskemi olup, özellikle preoperatuar kötü kondisyonu olan hastalarda intraoperatif seçilecek hipotermi yöntemi önemi kazanmaktadır.

Anahtar kelimeler: *Endotoksemi, hipotermi, kardiyopulmoner bypass.*

Endotoxin is a lipopolysaccharide originating from the cell wall of the bacteria with numerous potent pathophysiologic properties. It activates white blood cells and complement [1] and produces a spectrum of clinical syndromes ranging from fever to septic shock [2].

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Recent studies have indicated that endotoxemia occured at different levels during CPB [3]. Nowadays, it is believed that this adversely effects the posoperative recovery and the surgical results [4].

The purpose of the present study was to compare the effects of mild and moderate hypothermia on blood endotoxin levels.

MATERIAL and METHODS

Twenty patients undergoing CABG were included in the study (Table 1). Patients were enrolled between March-September 1995. The study was approved by the Institutional Commitee on Human Research, and appopriate informed consent was obtained from the patients. Patients who had signs of infections, leucocytosis and high sedimentation rates were excluded. Patients who had major abdominal operations, or had colon, intestine, or connective tissue disorders, abdominal aorta and mesenteric artery diseases were also excluded. Antibiotic prophylaxis was done by using Cefazolin. All patients received Diazepam, Morphine, and Scopolamin for premedication. Anaesthesia was induced with Fentanyl and Pancuronium and maintained with Isoflurane and Morphine. Nonpulsatile centrifugal pump was used during CPB (Delphin 7850 3M, Michigan, USA). During cross-clamping, the pump flow was 2,4 l/min per square meter body surface area. A membrane oxygenator was used for each patient (Dideco D703 Compact flow system). The hematocrit levels were kept at 20-25% levels intraoperatively. St. Thomas crystalloid cardioplegia was given through the aortic root for the myocardial protection and iced Ringer slush was applied for topical cooling. A myocardial temperature probe (Mallinkrodt, St. Louis, Missouri) was used to keep the temperature between 15-20°C during the cross-clamping of the aorta.

The patients were seperated into two equal groups of 10 patients. Moderate hypothermia (24-28°C) was applied to the patients in the first group, mild hypothermia (32-34°C) to the others.

Blood sampling: All blood samples were obtained in an aseptic fashion using endotoxin-free silicon-coated tubes (Terumo Venoject, Europe N.V. 3001 Leuven, Belgium). Blood cultures of all patients were obtained preoperatively

and postoperatively. A Swan-Ganz pulmonary artery catheter (Baxter Healthcare Corp. Edwards D.V. Irvine, Calif.) was introduced to obtain blood from the pulmonary artery. Blood sampling was performed during the following intervals: 1) at the prebypass period, 2) during aortic cross-clamping, 3) 20 minutes after the removal of the aortic cross-clamp, 4) immediately after the CPB, 5) at the postoperative 24th hour.

Measurement of endotoxin levels: Endotoxin levels were measured with non-chromogenic, semiquantitative LAL test (Sigma Chemical Company, St. Louis, USA). E-TOXATE is prepared from a lysate of circulating amebocytes of the horseshoe crab, Limulus Polyphenius. When exposed to minute quantities of endotoxin (lipopolysaccharides from the walls of gram-negative bacteria) the lysate increases in opacity as well as viscosity and may gel depending on the concentration of endotoxin. This principle of this test involves two steps; 1) endotoxin in the presence of calcium ions activates a trypsin-like [5], preclotting enzyme(s) [6], 2) the activated enzyme(s) modify a coagulation by limited proteolysis to produce a clottable protein [5]. E. Coli 055: B5LPS was used as the standard lipopolysaccharide. The concentrations of the endotoxins in the samples were calculated from a twofold dilution of the standard lipopolysaccharide. All the patients' sera were studied after inactivating the plasminogen inhibitors by 80°C water bath for 10 minutes. All the sera were studied twice for endotoxin determination to overcome the possible troubles in evaluation of the presence of a hard gel. The sensitivity of this assay was 0,05 EU/ml.

Statistical Analysis: Normally distributed, continuously variable data (e.g.endotoxin levels, the patient's age, duration of CPB...) were analyzed with the unpaired t test. Binary or categorically coded data (e.g.diabetic history, class...) were analyzed with the chi-square test. All differences were considered significant when p<0,05.

RESULTS

The duration of CPB did not differ significantly between Group 1 (46 to 163 minutes, 114 ± 36 minutes) and Group 2 (50 to 165 minutes, 107,8 ± 42,0 minutes). Aortic cross-clamping time also did not differ significantly between Group 1 (18 to 105 minutes, 65,9 ± 25 minutes) and Group 2 (20 to 97 minutes, 59,6 ± 31 minutes).

The mean rectal temperature was 26,8 ± 1,2°C in Group 1 and 33,8 ± 0,8°C in Group 2 (Table 2).

One patient from Group 1 died in ICU two hours postoperatively because of a low cardiac output. The other 19 patients had no significant postoperative complications. All of the blood cultures were negative. Endotoxins were not found in any of the priming fluids.

Table 1. Demographics of the patients

	GROUP I (n=10)	GROUP 2 (n=10)
F/M	1/9	2/8
Age (year)	57,7 ± 9	55,4 ± 7
NYHA Class 2 (%)	3 (30%)	2 (20%
NYHA Class 3 (%)	5 (50%)	6 (60)
NHYA Class 4(%)	2 (20%)	2(20%)
MI (%)	3 (30%)	4 (40%)
DM (%)	4 (40%)	3 (30%)
HT	6 (60%)	3 (30%)

Values are expressed in terms of mean ± standard deviation
MI: Myocardial Infarction, DM: Diabetes Mellitus, HT: Hypertension, F: Female, M: Male NYHA: New York Heart Association
Differences between groups were uniformly p > 0,05.

Table 2. Operative data of the patients

	GROUP 1 (n=10)	GROUP 2 (n=10)
BSA (m2*)	1,9±0,14	1,8±0,15
AORTIC CROSS CLAMP (min)*	65,9±25	59,6±31
TOTAL CPB TIME (min)*	114±36	107,8±42
ANASTOMOSIS*	2,5±0,9	2,4±1
HEMATOCRIT (%)*	22,2±1,8	21,8±1,3
RECTAL TEMPERATURE	26,8±1,2	33,8±0,8
PERFUSION PRESSURE (mmHg)*	57,3±6	57±5

Values are expressed in terms of mean ± standard deviation
**:p>0,05*

Changes in endotoxin levels (Figure 1): Patients have showed no endotoxemia before bypass. During aortic cross-clamping, 9 patients had positive endotoxins in Group 1 (2,5 to 5,0 EU/ml, 2,75 ± 1,4 EU/ml), 4 patients in Group 2 (0 to 2,5 EU/ml, 1,0 ± 0,4 EU/ml) (p<0,009). Immediately after the removal of aortic cross-clamp all of the patients had positive endotoxins in Group 1 (2,5 to 5,0 EU/ml, 4,5 ± 1,5 EU/ml) and 5 patients in Group 2 (2,5 to 5,0 EU/ml, 1,5 ± 1,0 EU/ml) (p<0,0002). Twenty minutes after the removal of aortic cross-clamp 9 patients had endotoxins in Group 1 (2,5 to 5,0 EU/ml, 3,0 ± 1,5 EU/ml) and 4 in Group 2 (o to 2,5 EU/ml, 1,0 ± 1,3 EU/ml) (p<0,0006). Immediately after CPB, 7 patients had positive endotoxins in Group 1 (2,5 to 5,0 EU/ml, 2,25 ± 1,5 EU/ml), 2 patients in Group 2 (0 to 2,5 EU/ml, 0,5 ± 1,0 EU/ml) (p<0,01). At the

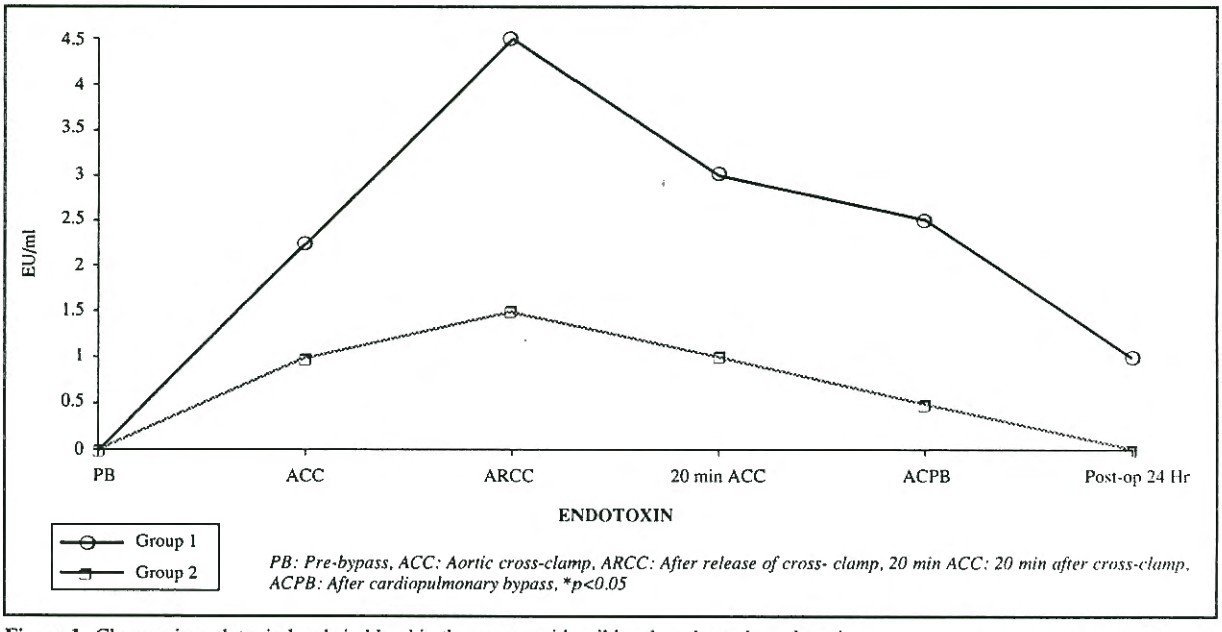

Figure 1: Changes in endotoxin levels in blood in the groups with mild and moderate hypothermia

postoperative 24th hour, 4 patients had endotoxins in Group 1 (0 to 2,5 EU/ml, 1,1 ± 1,0 EU/ml). No patients had endotoxins in blood in the second group ($p<0,01$).

DISCUSSION

Endotoxins are proteins consisting of lipopolysaccharides which take place on the outer membrane walls of gram-negative bacteria. Besides its own toxic effect, endotoxin activates all the septic cascade, mainly TNF-alpha (tumor necrotizing factor-alpha), IL-1, and PG E_2 and causes an inflammatory response in the whole body [7]. Sometimes, if the septic cascade cannot be reverted or neutralized, then the patient is evaluated as "sepsis syndrome" if the clinical criteria are met [8,9], and this continuous flow of the septic cascade may cause a spectrum of clinical conditions changing from multiple organ failure to death [9].

According to recent reports, endotoxin was detected in the blood at different stages during CPB. It was thought that the cause of this endotoxemia is endogenous. Totsuka and associates [10] have described three mechanisms responsible for the passage of the endotoxin into blood: 1) lymphatic absorption from the intestines via the portal vein and/or venous absorption, 2) direct passage through the portal vein from the intestines, 3) direct absorbtion from the peritoneal cavity.

Previous studies have showed that CPB causes splanchnic hypoperfusion [11,12,13]. Intestinal ischemia impairs the wall permeability [14] and causes the endotoxin to pass into the circulation. Pulsatile perfusion increases the splanchnic flow. Watarida and associates [15] showed that the endogenous endotoxin levels were lower with pulsatile perfusion. Lazenby and associates [16] have shown that, with constant flow index, splanchnic hypoperfusion occurred at 28°C, and lower temperatures.

It was recently stated that hypothermia might have been the cause of mucosal ischemia. Ohri and associates have measured blood flow with a laser Doppler and intramucosal pH (H_i) by using a tonometer in their experimental study. Especially during the rewarming period, a shunt would take place at the side of active mucosa and this would create a gradient between arterial and venous oxygen content. Consequently, a mucosal acidosis and ischemia of the villi would occur [11].

At 28°C and lower temperatures, the intestinal motility decreases and the intraluminal toxic materials will be absorbed [17].

In our study, the higher endotoxin level seen in moderately hypothermic group, can be explained by the information given above.

Normally, endotoxins in the circulation are detoxified by the Kupffer cells, which are parts of the reticuloendothelial system, with a L-arginine dependent mechanism [9]. Because of the depressive effects of hypothermia and CPB on the immune system and enzyme activity [18], this procedure may possibly not be accomplished. This can be a secondary mechanism for the production of endotoxemia.

No endotoxins were detected from samples of 5 patients in Group 2. We believe that this was not due to the absence of endotoxins, rather due to the very low level of endotoxin which was not detectable by the test itself. This may be due to the non-chromogenic assay used; in our study first dilution (1/10) inactivates the plasminogen inhibitors in the 80°C water bath.

In the present study endotxin levels peaked just after the removal of aortic cross-clamping in both groups. After the removal of the clamp, the heart is allowed to eject, and the higher systolic pressures achieved, would give rise to an increased flow through the superior mesenteric system. This increased flow would "flush out" endotoxins in the stagnated blood of the splanchnic system to the circulation [4].

In conclusion, endotoxin levels become higher when hypothermia gets deeper probably due to intestinal ischemia, decreasing intestinal motility and lower enzyme activity. The present study suggests that when hypothermia is the technique of choice, the deleterious effects of endotoxemia in patients with poor conditions must be considered.

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