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Responses of interleukin-6 and tumor necrosis factor during and after cardiac surgery undergoing cardiopulmonary bypass and pancreatoduodenectomy

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Abstract: To evaluate the effect of cardiopulmonary bypass on immunological function, we measured interleukin-6 (IL-6) and tumor necrosis factor (TNF) in 12 patients undergoing cardiac surgery during and after cardiopulmonary bypass, and in 10 patients with pancreatoduodenectomy. Plasma IL-6 levels were determined using the Human Interleukin 6 ELISA Kit, and TNF levels were determined using a highly sensitive sandwich enzyme immunoassay. In patients with cardiac surgery, plasma levels of IL-6 and TNF increased during cardiopulmonary bypass, and in patients with pancreatoduodenectomy, IL-6 and TNF levels significantly increased at the end of intraabdominal manipulation. These results suggest that endotoxin may have activated the immune system and stimulated cytokine production after pancreatoduodenectomy and during bypass.

Key words: Interleukin-6, Tumor necrosis factor, Cardiac surgery, Pancreatodoudenectomy

Introduction

It is reported that surgery and associated infection stimulate the production of endogenous mediators which affect the response of the host to injury in various organs [1]. Despite the diversity of injuries, including surgical, traumatic, and septic injuries, there are common immunological and biochemical changes [2]. Recently, increased serum levels of interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF) have been demonstrated in animals and humans in various forms of tissue injury and inflammation [3,4]. Cardiopulmonary bypass (CPB) has also been associated with a complex array of postoperative clinical side effects: coagulopathy, increased capillary permeability, fever, and end-organ dysfunction. It has been reported that immunological functions are more depressed after cardiac surgery than other forms of surgery [5,6]. In this study, we measured the plasma concentrations of IL-6 and TNF before, during, and after cardiac surgery undergoing CPB to evaluate the effect of cardiopulmonary bypass on immunological function. We also estimated the surgical stress of cardiac surgery by comparing the results with those of pancreatoduodenectomy.

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Materials and methods

Twelve patients (aged 32–64 years) scheduled for cardiac surgery under cardiopulmonary bypass and 10 patients (aged 55–77 years) scheduled for pancreatoduodenectomy were included in the study. The study protocol was approved by the ethical committee of Osaka Police Hospital and informed consent was obtained from each patient. Patients who had any immune or endocrine disorders were excluded from the study. None of the patients had infectious or renal disorders preoperatively. Patients were allocated into two groups under general anesthesia: group A consisted of 10 patients undergoing pancreatoduodenectomy, and group B consisted of 12 patients scheduled for cardiac surgery under CPB. Pancreatoduodenectomy was performed under general anesthesia with supplemental epidural analgesia up to the lower thoracic levels (T7–10). Atropine 0.5 mg or scopolamine 0.4 mg was administered intramusculary as a preanesthetic 1 h before surgery. On arrival at the operating room, radial and pulmonary artery catheters were inserted using local anesthesia in group B. General anesthesia was induced with midazolam 0.3 mg/kg, and vecuronium 0.1 mg/kg was administered for neuromuscular blockade to facilitate endotracheal intubation. In group A patients, appropriate levels of epidural analgesia were

obtained using an intermittent administration of 1.5% lidocaine. The analgesic levels were confirmed by the loss of sensation to cold stimuli before the induction of general anesthesia. Anesthesia was maintained with an end-tidal concentration of isoflurane of 1–1.5% for pancreatoduodenectomy, and with fentanyl 20$\mu g \cdot kg^{-1} \cdot h^{-1}$ for cardiac surgery. Nitrous oxide 50% was supplemented in group A patients to maintain general anesthesia. In patients with cardiac operation, pure oxygen was given during general anesthesia. Electrocardiogram was continuously monitored using a standard II lead and/or a V5 lead. In patients with cardiac surgery, 3 M Total Bypass Membrane Oxygenator with polypropylene membrane (JOSTRA, Hirrlingen, Germany) was used for cardiopulmonary bypass. Synchronized roller pumps (PMO-15-200, JOSTRA, Hirrlingen, Germany) were utilized for the return of blood to the oxygenator and to the aortic cannula. CPB circuits were primed with 2000 ml of lactated Ringer's solution and 500 ml of albumin. Packed red cells were transfused as needed to maintain the hematocrit above 20% during CPB. Moderate hypothermia (27°C) was employed routinely. After aortic cross-clamping, cardioplegia was infused via the aortic root. The estimated pump flow rates were 3 $L \cdot min^{-1} \cdot m^{-2}$ of body surface area at normothermia, and 2.4 $L \cdot min^{-1} \cdot m^{-2}$ at 27°C. The perfusion pressure during CPB was maintained at about 60 mmHg. At the termination of CPB, patients were rewarmed to 37°C. In patients with pancreatoduodenectomy, epidural administration of lidocaine was discontinued at 3 days after surgery. Blood samples were collected as follows.

T1, the day before surgery; T2, immediately after skin incision T3, 4 h after skin incision (during CPB in cases of cardiac surgery); T4, before skin closure; T5, on the 1st postoperative day (POD); T6, on the 3rd POD; and T7, on the 5th POD.

Blood samples were drawn through an indwelling catheter inserted into a vein and collected in prechilled tubes containing ethylenediaminetretraacetate (EDTA). After centrifugation at 4°C, separated plasma samples were stored at −20°C until the assay. Plasma IL-6 levels were determined using the Human Interleukin 6 enzyme-linked immunoadsorbent assay (ELISA, Toray Fuji Bionix Inc, Tokyo, Japan) Kit [7]. The ELISA was performed by an avidin-biotin amplified two-step sandwich method. Each well of the antibody-coated plate was washed with 400 μl of washing buffer, and 50 μl of assay buffer and 100 μl of sample were dispensed into wells and the plate was incubated at 25°C for 2 h. The plate was shaken on a microplate mixer throughout the incubation time. After the wells had been washed three times with 400 μl of washing buffers, 100 μl of biotin-labeled IC67F(ab')2 antibody, 100 μl of avidin-HRP complex and 100 μl of substrate solution were sequentially reacted at 25°C for 1 h, 0.5 h, and 1 h, respectively, with a washing step between each incubation. The reaction was stopped by adding 100 μl of 4.5 N H_2SO_4 and the optical density at 490 nm was measured using a microplate reader. The minimal detectable value of this method was 15 pg of IL-6 ml^{-1} plasma. Plasma TNF levels were determined using a highly sensitive sandwich enzyme immunoassay [8].

Comparison of IL-6 and TNF levels were performed by the repeated measures analysis of variance (ANOVA) with Student's *t*-test. Student's *t*-test or the Mann-Whitney test was used for demographic or other data. $P < 0.05$ was considered as significant. Values are expressed as the mean ± standard deviation (SD) of each group.

Results

Table 1 shows the age, operation and CPB time, intraoperative blood loss and transfusion in all 22 patients. There were no significant differences in either the age or intraoperative blood loss between the groups. The operation time was significantly greater in group A than in group B. Table 2 and Fig. 1 show the time course of plasma IL-6 levels before, during, and after surgery. In group A patients, IL-6 levels were significantly

Table 1. Details of patient background

	PD	CS	*P*
Number of patients	10	12	
Age (years)	55.5 (4.4)	63.3 (2.4)	n.s
Operation time (min)	558.5 (100.9)	374.2 (25.4)	<0.01
CPB time (min)		122.8 (9.2)	
Blood loss (ml)	1027.0 (116.1)	1517.6 (229.4)	n.s.
Transfusion (ml)	738.0 (119.2)	1785.0 (273.7)	<0.05

Values are mean (SD).
PD, pancreatoduodenctomy; CS, cardiac surgery.

Table 2. Changes of serum levels of interleukin-b

	Group A (pg/ml)	Group B (pg/ml)
T1	21.4 ± 9.7	47.5 ± 32.8
T2	15.9 ± 6.6	78.2 ± 64.7
T3	16.0 ± 4.2	136.1 ± 54.6**
T4	251.8 ± 94.6*	129.9 ± 85.3**
T5	159.4 ± 84.1*	80.1 ± 56.1
T6	65.2 ± 48.9	47.7 ± 31.9
T7	32.6 ± 17.0	35.1 ± 19.3

Values are mean ± SD.
T1, the day before surgery; T2, immediately after skin incision; T3, 4 h after skin incision in group A or during CPB in group B; T4, before skin closure; T5, on the 1st POD; T6, on the 3rd POD; T7, on the 5th POD.
* $P < 0.01$ VS. T1.
** $P < 0.01$ VS. T1.

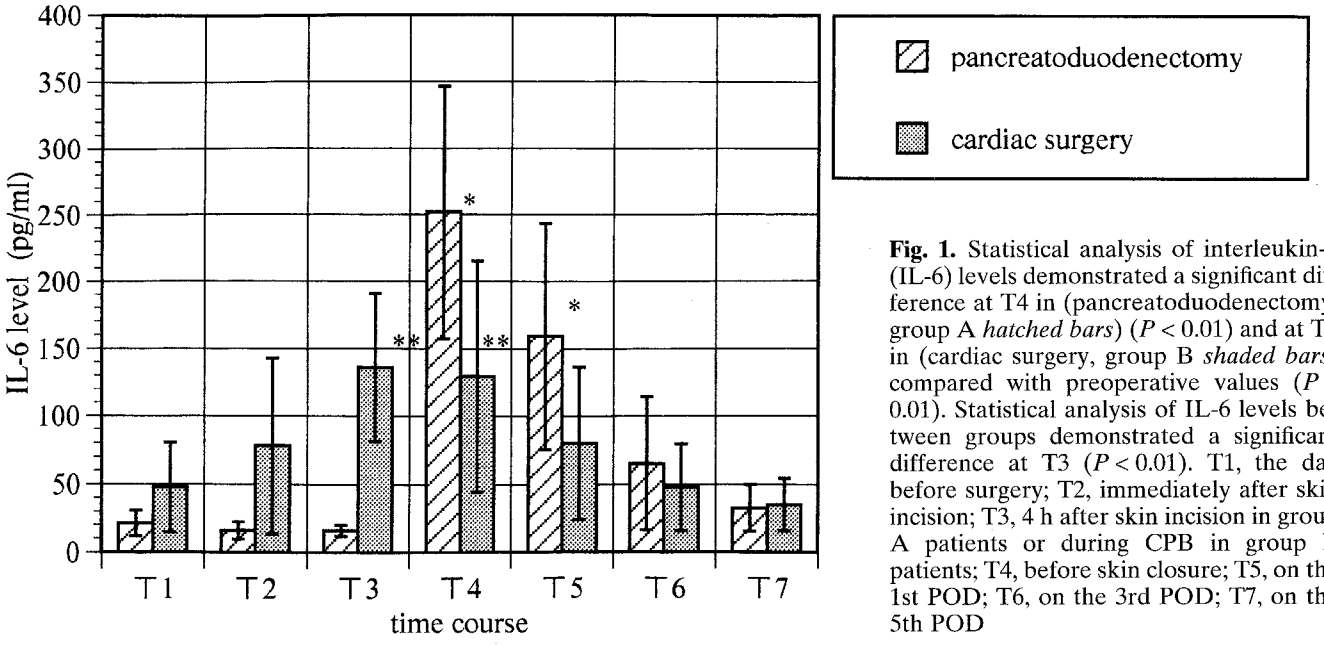

Fig. 1. Statistical analysis of interleukin-6 (IL-6) levels demonstrated a significant difference at T4 in (pancreatoduodenectomy, group A *hatched bars*) ($P < 0.01$) and at T3 in (cardiac surgery, group B *shaded bars*) compared with preoperative values ($P < 0.01$). Statistical analysis of IL-6 levels between groups demonstrated a significant difference at T3 ($P < 0.01$). T1, the day before surgery; T2, immediately after skin incision; T3, 4 h after skin incision in group A patients or during CPB in group B patients; T4, before skin closure; T5, on the 1st POD; T6, on the 3rd POD; T7, on the 5th POD

higher at T4. In group B patients, IL-6 levels were higher at T3 compared with presurgery levels. There was a significant difference between the two groups at T3. The maximum levels were attained at T4 in group A and at T3 in group B. On the 3rd POD, there were no significant diferrences in either group compared with the presurgery levels. IL-6 levels at 4 h after skin incision were 16 ± 4.2 pg and 136 ± 5.46 pg in groups A and B, respectively.

Table 3 and Fig. 2 show the TNF levels before, during, and after cardiac surgery or pancreatoduodenectomy. TNF levels significantly increased at T4 and at T3 compared with presurgery levels in groups A and B, respectively. The maximum levels were attained on the 3rd POD in group A and during CPB in group B. On the 1st POD, there was no significant difference compared with presurgery level in group B.

Discussion

Surgical injury induces the local inflammatory reaction as well as the stress response of the immune and hematopoietic systems. These responses are associated

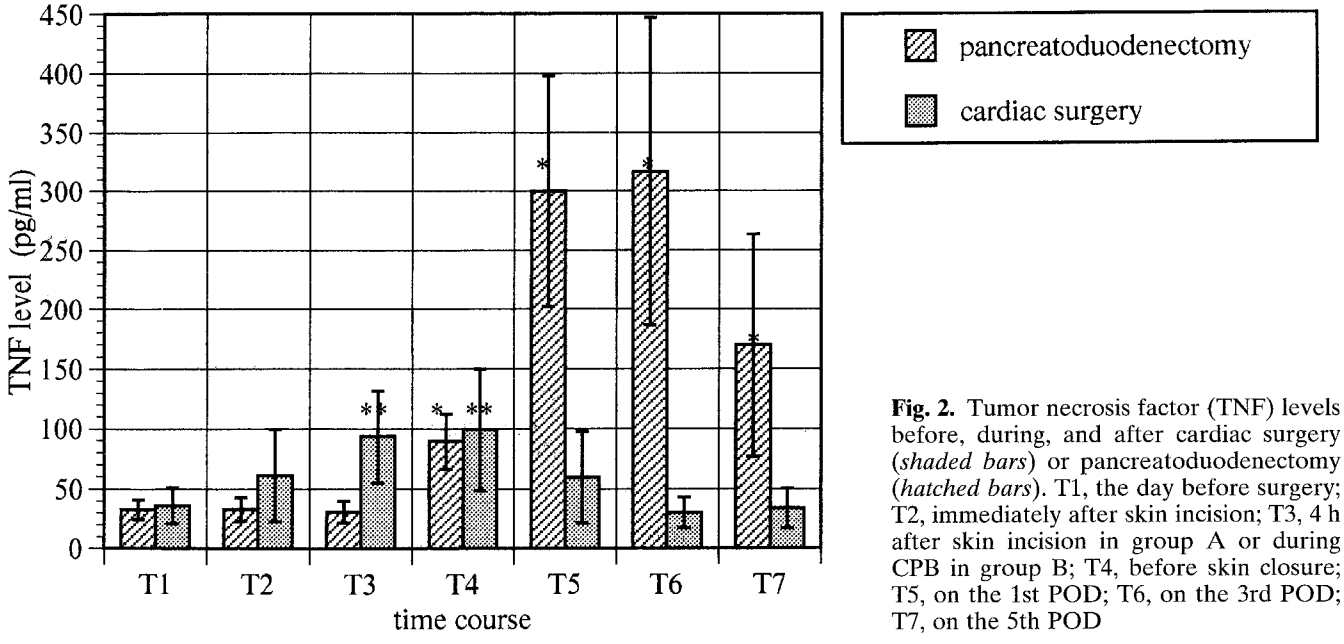

Fig. 2. Tumor necrosis factor (TNF) levels before, during, and after cardiac surgery (*shaded bars*) or pancreatoduodenectomy (*hatched bars*). T1, the day before surgery; T2, immediately after skin incision; T3, 4 h after skin incision in group A or during CPB in group B; T4, before skin closure; T5, on the 1st POD; T6, on the 3rd POD; T7, on the 5th POD

Table 3. Changes of serum levels of tumor necrosis factor

	Group A	Group B
T1	32.7 ± 8.4	36.1 ± 14.9
T2	32.9 ± 10	60.8 ± 38.2
T3	30.9 ± 9.1	93.3 ± 38.5**
T4	89.2 ± 22.9*	99.0 ± 50.6**
T5	299.7 ± 97.8*	59.3 ± 37.9
T6	316.3 ± 130.4*	30.2 ± 12.8
T7	169.5 ± 93.4*	33.7 ± 16.6

Values are mean *.006 SD.
T1, the day before surgery; T2, immediately ofter skin incision; T3, 4 h after skin incision in group A and during CPB in group B; T4, before skin closure; T5, on the 1st POD; T6, on the 3rd POD; T7, on the 5th POD.
* $P < 0.01$ VS. T1.
** $P < 0.01$ VS. T1.

with increased production of a variety of endogenous mediators. TNF is a cytokine that is known to be released from macrophages after endotoxin administration. TNF is a major mediator of endotoxin toxicity and its in vivo administration produces effects similar to those of endotoxin [9,10]. Recent studies have shown that some physiologic effects of endotoxin are regulated by the release of endogenous mediators from immune cells such as IL-1, IL-6 [11], and TNF. It is reported that endotoxin stimulates the production of TNF and then IL-6 [12,13]. However, there are few reports [6,14] concerning serum IL-6 during and after open heart surgery. In the present study, we measured IL-6 and TNF in 12 patients undergoing cardiac surgery during and after CPB and demonstrated increased levels of IL-6 and TNF during CPB.

Cardiac surgery with CPB is generally associated with comparable or even greater stress compared with radical operation for esophageal cancer or pancreatoduodenectomy. We intended to estimate the approximate surgical stress of cardiac surgery and pancreatoduodenectomy by comparing plasma cytokine concentrations in both groups, hypothesising that surgical stress is, to a certain degree, reflected in the plasma cytokine concentrations. The present results show that plasma cytokine concentrations tended to be higher in group B than in group A during surgery, indicating that the intensity of surgical stress during cardiac surgery is much greater than that during pancreatoduodenectomy. However, postoperatively, plasma cytokine concentrations tended to be higher in group A than in group B. One possible explanation for this marked increase in plasma cytokine concentrations after pancreatoduodenectomy is as follows. Because pancreatoduodenectomy exerts surgical manipulation directly on the intestine and because it involves sections and anastmoses of the gastrointestinal tract, more endotoxin would derive from the intestinal flora into the systemic circulation and induce cytokine production compared with that observed after cardiac surgery. Besides the plasma cytokine data, we must take various factors such as operation technique, intraoperative bleeding, and operation time into consideration when estimating surgical stress. So only from our current data, we should be cautious about making any predictions about surgical stress in both groups.

In the present study, IL-6 levels were increased during CPB in group B. Lahat et al. [6] reported elevated serum levels of IL-6, IL-1, and TNF in patients undergoing coronary artery bypass graft in nine patients, and Sakai et al. [14] measured the serum levels of IL-6 and α-MSH in 12 patients undergoing heart transplantation. They reported that the serum level of IL-6 decreased transiently at the initiation of CPB and that the IL-6 release was augmented significantly when the patients were weaned from CPB [14]. Their results are consistent with our results. In the present study, no significant change was found in IL-6 and TNF after skin incision in the prebypass period. This absence of an initial response may be due to the limited time interval between skin incision and initiation of CPB in this study, since the distribution half-life and elimination half-life of IL-6 are 3 min and 55 min, respectively. Cardiac operation can result in changes in cell-mediated immunity and depress immunological functions [5,15,16]. As for the transient decrease in the IL-6 level at the start of CPB reported by Sakai et al. [14], there may have been some immunodepression related to CPB as well as participation of hypothermia and hemodilution.

Naito et al. [12] demonstrated that TNF-α and IL-6 increased during and after pancreatoduodenectomy in 13 patients and that cytokine levels did not change in 10 patients undergoing unilateral total hip replacement. Their results suggest that the increased IL-6 level was not due to the local inflammation that follows surgery. It is reported that endotoxin which appeared in the systemic circulation stimulated the production of TNF and afterwards stimulated IL-6 [12,13]. Their report on IL-6 levels is consistent with our results. William et al. [17] measured the circulating endotoxin levels during pediatric cardiac surgery and reported that endotoxin was detected in the blood of 16 (67%) of the 21 patients. (The majority of the samples positive for endotoxin were withdrawn during CPB. [17]) They speculated that a possible source of endotoxin may be the patient's intestinal flora; decreased mean arterial pressure and altered blood distribution during CPB may impair perfusion to the intestine and affect mucosal permeability [17]. Also, in our present data, the TNF level increased during CPB and this finding does not conflict with the appearance of endotoxin in the systemic circulation during CPB. In group A, the TNF level did not change

at T3 and markedly increased at T4. We speculate that the marked increase in the plasma TNF level at T4 was due to the direct manipulations on intestine and because the time interval of 4 h might be too short to detect a significant increase in the plasma TNF level in group A.

In conclusion, we demonstrated increased levels of IL-6 and TNF during CPB in cardiac surgery. These results suggest that during CPB, endotoxin may derive from intestinal flora due to the impaired perfusion to the intestine and stimulate cytokine production. Compared with group A, plasma cytokine levels in group B tended to be higher during surgery, while after surgery those in group A tended to be higher. However, only from our current data we should be cautious about estimating the intensity of the stress response in cardiac surgery in comparison with that in pancreatoduodenectomy.

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