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Fibrinolytic shutdown after cardiopulmonary bypass surgery is caused by circulating cytokines during operation, accompanied by endothelial injury

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Abstract: It has been hypothesized that increased cytokines during cardiopulmonary bypass surgery cause postoperative fibrinolytic shutdown. To investigate the role of cytokines and to elucidate its mechanism, tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), plasminogen activator inhibitor-1 antigen (PAI-1 Ag), PAI-1 activity, and thrombomodulin in 16 patients undergoing elective cardiopulmonary bypass surgery were analyzed after induction of anesthesia, before and after cardiopulmonary bypass, and at the end of the operation. During surgery, TNF-α and LI-1β were detected in 44% and 63% of the cases, respectively. PAI-1 Ag ($P < 0.01$), PAI-1 activity ($P < 0.001$) and thrombomodulin ($P < 0.01$) were significantly increased at the end of the operation. The patients were divided into two groups and examined: Group 1 with either cytokine detected ($n = 12$); and group 2 without detection of cytokines ($n = 4$). At the end of the operation, both PAI-1 Ag ($P < 0.05$) and PAI-1 activity ($P < 0.01$) were significantly increased in group 1 compared with group 2. In group 1, there was a significant positive correlation between thrombomodulin and PAI-1 Ag ($r^2 = 0.117$, $P < 0.05$) and PAI-1 activity ($r^2 = 0.124$, $P < 0.05$). In conclusion, TFN-α and IL-1β were released into the systemic circulation during cardiopulmonary bypass surgery, and this release may have been caused by vascular endothelial injury. These cytokines increased PAI-1 activity.

Key words: Cardiopulmonary bypass surgery, Cytokine, Plasminogen activator inhibitor-1, Thrombomodulin

Introduction

Endotoxin induces endothelial cell damage [1] resulting in an increase in plasminogen activator inhibitor-1 (PAI-1) [2,3]. The monocyte and macrophages stimulated by endotoxin produce interleukin-1β (IL-1β) and tissue necrosis factor-alpha (TNF-α). These cytokines also cause endothelial cell perturbation, leading to an increase in PAI-1 [3–7]. Cytokines also affect the synthesis of thrombomodulin and its relaease into the circulation [8–10].

During cardiopulmonary bypass surgery, endotoxin and TNF-α are released into the systemic circulation [11,12]. However, agreement has not been reached concerning changes in IL-1 [13,14] and PAI-1 [15,16] during cardiopulmonary bypass surgery, and the relationship between TNF-α or IL-1β and PAI-1 has never been adequately elucidated. PAI-1 induce postoperative deep vein thrombosis and pulmonary embolism [3,16].

We hypothesized that TNF-α or IL-1β, released into the systemic circulation during cardiopulmonary bypass surgery, would cause endothelial cell injury and then promote the synthesis and release of PAI-1 from the endothelium. To test this hypothesis, we determined the levels of TNF-α, IL-1β and PAI-1 during cardiopulmonary bypass surgery and then examined their relationship. Circulating thrombomodulin was used as a marker of vascular endothelial cell injury [10].

Patients and methods

Patients

This study protocol was approved by the Ethical Committee of the Sapporo City General Hospital with no exclusion criteria. The study involved 16 patients without any liver or renal dysfunction who were scheduled to undergo elective cardiopulmonary bypass surgery. The operations included a coronary artery bypass graft in nine cases, a valve replacement in six, and an atrial septal defect patch closure in one.

The patients were divided into two groups: Group 1, those who tested positive for either or both cytokines ($n = 12$), and group 2, who were negative ($n = 4$).

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Perioperative management

As premedication, scopolamine 0.3–0.5 mg and morphine hydrochrolide 0.1 mg·kg^{-1} were given intramuscularly. All the Patients were anesthetized with high-dose fentanyl combined with midazolam. Vecuronium bromide was used as a muscle relaxant, and the patients were ventilated with 50% oxygen in air. Cardiopulmonary bypass circulation was performed with a roller pump (7400 MDX, Sarns, Annarbor, Michigan, USA) and membrane oxygenator (SMO II, Sarns) at 2.0–2.4 L·m^{-2} of non-pulsatile flow. Moderate total body hypothermia was used during the perfusion. Heparin used was 2 mg·kg^{-1}, and supplemental injections were administered so that the activated coagulation time could be maintained at more than 400 s. Protamine was administered at 1–1.5 times the dose of heparin.

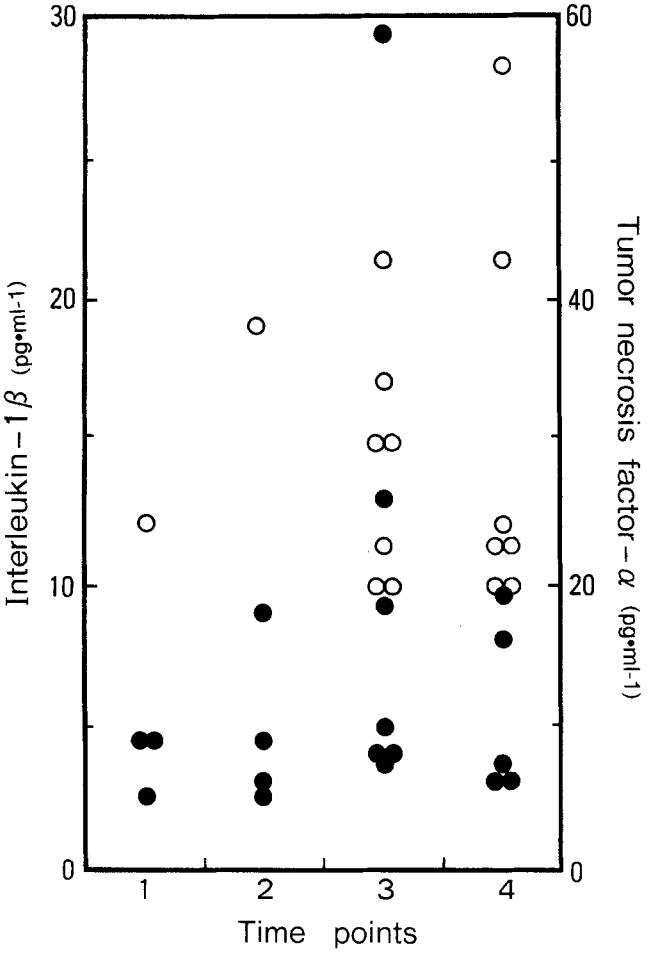

Fig. 1. The detection of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in patients undergoing cardiopulmonary bypass surgery. *Closed* and *open circles* denote TNF-α and IL-1β, respectively. At least one cytokine was positive in 75% of the cases. 1, after induction of anesthesia; 2, before cardiopulmonary bypass; 3, after cardiopulmonary bypass; 4, at the end of the operation

Blood sampling and measurements

Blood samples were taken via a catheter inserted into the radial artery: (1) Immediately after induction of anesthesia, (2) before cardiopulmonary bypass (before administration of heparin), (3) at the end of cardiopulmonary bypass (before administration of protamine), and (4) at the end of the operation. The withdrawn blood was put into two prechilled tubes, and centrifuged at 3000 rpm for 10 min at 4°C. The resultant serum and plasma were stored at −80°C until measurement. The parameters, the methods for measurement, and the values of normal adult controls were as follows: serum IL-1β [Immunoradiometric assay, IL-1β-IRMA, MEDGENIX DIAGNOSTICS Brussels, Belgium, less than 5 pg·ml^{-1}]; serum TNF-α [enzyme-linked immunosorbent assay (ELISA), HUMAN TNF-α ELISA LIT, Otsuka pharmacoutical, Tokushima, Japan, less than the limits of detection], plasma PAI-1 antigen concentration (PAI-1 Ag) [ELISA, PAI-1 ELISA kit, Momozyme Hoersholm, Denmark, less than 50 ng·ml^{-1}]; plasma PAI-1 activity [Spectrolyse/pL (V1-1), Bipool Umeå, Sweden, less than 5.5 U·ml^{-1}]; and serum thrombomodulin [one-step sandwich enzyme immunoassay (EIA), TM Panacela, Fuji Revio Tokyo, Japan, less than 4 ng·ml^{-1}].

Statistical analysis

The values are expressed as mean ± SEM. The changes of the above parameters with time were analyzed for variance with repeated measures following Scheffe's multiple comparison test. The comparison between the two groups was made using the Mann-Whitney U-test and chi-square test. Any correlations were examined using a linear regression by the least squares method. $P < 0.05$ was considered statistically significant.

Results

IL-1β and TNF-β were detected in ten (62.5%) and seven cases (43.8%), respectively. Both of the cytokines were positive in five (31.3%), Figure 1 shows the points of detection and the data. Many of the cases showed positive tests for both cytokines during cardiopulmonary bypass. PAI-1 Ag, PAI-1 activity, and thrombomodulin were all significantly increased following cardiopulmonary bypass (Table 1).

The perioperative data for the patients, as seen in Table 2, did not show any significant differences between these two groups. At the end of the operation, the levels of PAI-1 Ag (222.8 ± 58.7 *vs* 72.2 ± 9.6 ng·ml^{-1}, $P < 0.05$) and PAI-1 activity (74.3 ± 14.1 *vs* 21.9 ± 2.2 U·ml^{-1}, $P < 0.01$) were significantly increased in

Table 1. Successive changes in plasminogen activator inhibitor-1 and thrombomodulin during cardiopulmonary bypass surgery

	1	2	3	4	
PAI-1 Ag ($ng \cdot ml^{-1}$)	70.5 ± 10.8	74.0 ± 8.2	76.3 ± 5.3	185.1 ± 46.7*	$P < 0.01$
PAI-1 activity ($U \cdot ml^{-1}$)	6.2 ± 0.9	6.3 ± 0.8	2.5 ± 0.7	61.2 ± 12.0*	$P < 0.001$
Thrombomodulin ($ng \cdot ml^{-1}$)	1.7 ± 0.1	1.7 ± 0.1	2.2 ± 0.4	3.9 ± 0.8*	$P < 0.01$

PAI-1 Ag, plasminogen activator inhibitor-1 antigen; 1, after induction of anesthesia; 2, before cardiopulmonary bypass; 3, at the end of the cardiopulmonary bypass; 4, at the end of surgery.
* $P < 0.05$ *vs* 1, 2, and 3.

Table 2. Perioperative patient data

	Cytokines present	Cytokines absent	*P* value
Age (years)	58 ± 2	55 ± 3	NS
Sex (male/female)	6/6	2/2	NS
Anesthesia time (min)	458 ± 32	431 ± 28	NS
Surgery time (min)	401 ± 30	365 ± 29	NS
Bypass time (min)	173 ± 16	170 ± 10	NS
Aortic cross clamp time (min)	118 ± 12	95 ± 10	NS
Urine output (ml)	2215 ± 196	2577 ± 225	NS
Heparin (mg)	180 ± 10	194 ± 9	NS
Protamine (mg)	192 ± 14	237 ± 6	NS
Activated coagulation time (sec) 1	145 ± 5	145 ± 6	NS
2	523 ± 31	566 ± 44	NS
3	137 ± 3	158 ± 12	NS

1, before cardiopulmonary bypass; 2, during cardiopulmonary bypass; 3, after cardiopulmonary bypass.

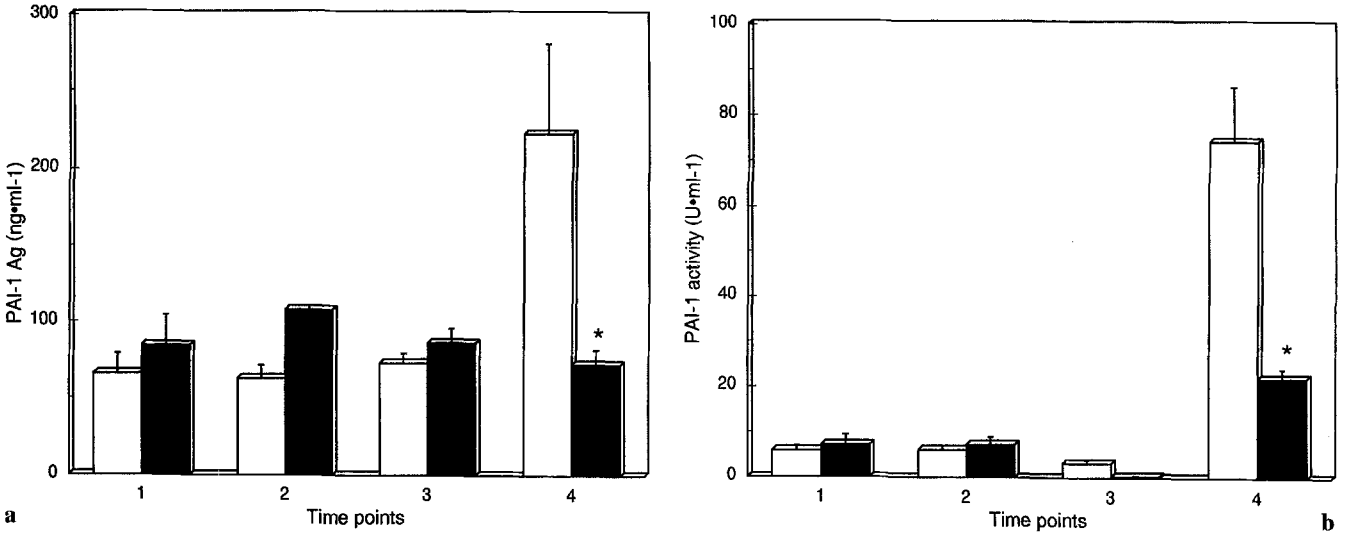

Fig. 2. a Changes in plasminogen activator inhibitor-1 antigen (pAI-1 Ag) during cardiopulmonary bypass surgery. *White*, cytokines present; *black*, cytokines absent. In group 1, PAI-1 Ag was markedly increased after the end of the cardiopulmonary bypass. **b** Changes in PAI-1 activity during cardiopulmonary bypass surgery. This activity was found to be prominently enhanced after the end of cardiopulmonary bypass. This increase was significantly greater in group 1 with cytokines than in group 2. 1, after induction of anesthesia; 2, before cardiopulmonary bypass; 3, after cardiopulmonary bypass; 4, at the end of the operation. *$P < 0.01$ *vs* group 1

group 1 (Fig. 2). On the contrary, at time point 4 the levels of thrombomodulin tended to be higher in group 2 (2.9 ± 0.6 *vs* 6.8 ± 2.6 ng·ml^{-1}). A significant positive correlation was found between thrombomodulin and PAI-1 Ag ($y = 34.8x + 33.2$, $r^2 = 0.117$, $P < 0.05$) or PAI-1 activity ($y = 11.2x + 1.13$, $r^2 = 0.124$, $P < 0.05$) in group 1.

Discussion

During cardiopulmonary bypass surgery [11,12,14] as well as major abdominal surgery [17], endotoxin is released into the systemic circulation and this leads to the formation of TNF-α during the operation. Although we did not determine the endotoxin, TNF-α was detected in 44% of the cases, which was nearly consistent with the report by Casey et al. [12], in which both endotoxin and TNF-α were simultaneously measured. IL-1β has not been measured [11,12] or detected even if determined [14,17]. Haeffner-Cavaillon et al. [13] reported the induction of IL-1β production in patients undergoing cardiopulmonary bypass surgery, the cause of which they attributed to complement activation, whereas they could not demonstrate the release of TNF-α. The discrepancies between these reports may be caused by the differences in the methods of determination or the sensitivity of the kits used for measurements. In our study, IL-1β was detected in 63% of the cases, while TNF-α and IL-1β were first detected at the same time during cardiopulmonary bypass surgery in 31%. Although the elimination half-life of the TNF-α and IL-1β in the circulation is very short, and since it can be ruled out that we missed peaks of increasing cytokines, the results suggest that the release of endotoxin and subsequent complement activation with cardiopulmonary bypass surgery might stimulate production of TNF-α and IL-1β. Further, cytokines could be detected immediately after the induction of anesthesia and before the start of cardiopulmonary bypass, which are similar to other reported findings [11,12,17]. It is considered that the release stimuli might include pyrogens present in the fluids administered to the patients, endotoxin contamination of the perfusion circuit, or anesthesia and/or surgical stress itself.

The stimuli, such as endotoxin [2,3], TNF-α, IL-1β [3–7], and thrombin [3,18], perturb endothelial cells, leading to increased synthesis, cell surface expression, and finally the release into the systemic circulation of PAI-1. The PAI-1 determined in our study was derived not from the platelets but from the endothelial cells [19]. Our results that both PAI-1 Ag and PAI-1 activity were significantly higher in group 1 than in group 2 together with the above-mentioned observations suggest that TNF-α or IL-1β might contribute to fibrinolytic shutdown [16] after cardiopulmonary bypass surgery. In group 2, only PAI-1 activity was slightly increased after cardiopulmonary bypass. We measured high levels of fibrinopeptide A, indicating a large amount of thrombin formation during cardiopulmonary bypass surgery [20]. Thus, thrombin or other unknown factors seemed to be responsible for the increase in PAI-1 activity seen in group 2.

Cellular thrombomodulin is an endothelial cell membrane protein that is a cofactor required for the rapid activation of plasma protein C. Circulating thrombomodulin, we determined, is not secreted by the endothelial cells but rather is cleaved from vascular (cellular) thrombomodulin with loss of part of the *O*-glycosylation site-rich region, the transmembrane domain, and the cytoplasmic tail due to endothelial cell damage, and then released into the circulation [8,10]. Circulating thrombomodulin is reported to increase in patients with diseases that cause vascular endothelial cell damage such as Thrombotic Thrombocytopenic Purpula (TTP), Disseminated Intravascular Coagulation (DIC), and adult respiratory distress syndrome (ARDS), and has been clinically applied as a new molecular marker of the degree of damage to the vascular endothelial cells [10]. Our data, which demonstrated an increase in circulating thrombomodulin, indicate or demonstrate or suggest that endothelial cell injury occurs during cardiopulmonary bypass surgery. The causes of such damage appear to include endotoxin, TNF-α, IL-1β, and neutrophils activated by these factors [21]. Unlike PAI-1, the increase in thrombomodulin tended to be smaller in group 1. This might be because cytokines reduced the synthesis of thrombomodulin and its cell surface expression [9].

Increased PAI-1 production was accompanied by endothelial cell injury [3,18], while TNF-α and IL-1β caused microvascular injury [21]. The increase in PAI-1 in group 1 was pronounced while there was a significant positive correlation of PAI-1 Ag and PAI-1 activity with thrombomodulin. These results suggest that endothelial cell injury might lead to the release of PAI-1 during cardiopulmonary bypass surgery.

TNF-α and IL-1β induced systemic inflammatory reactions and hemodynamic instability following surgery [11–13]. PAI-1 is a risk factor of postoperative deep venous thrombosis and pulmonary embolism [3,16]. We demonstrated that these three factors, associated with the vascular endothelial cell damage, could be simultaneously detected during cardiopulmonary bypass surgery. Although the present study did not investigate the postoperative morbidity, it seems likely that the presence and extent of the release of cytokines or PAI-1 may exert a great influence on the postoperative morbidity. Further studies are required to clarify this point.

In conclusion, we demonstrated that TNF-α, IL-1β, PAI-1, and circulating thrombomodulin appeared in the systemic circulation during cardiopulmonary bypass surgery. The synthesis and release of PAI-1 were at least partly caused by TNF-α and IL-1β. Vascular endothelial cell injury might give rise to these changes.

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