Alterations in Biomarkers of Endothelial Function Following On-Pump Coronary Artery Revascularization

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Background: Cardiopulmonary bypass (CPB) has been associated with activation and injury of endothelial cells, probably responsible for the systemic inflammatory response syndrome (SIRS) taking place in these patients. **Methods:** We measured plasma concentrations of soluble P-selectin (sP-s), E-selectin (sE-s), tetranectin (TN), vonWillebrand factor (vWF) levels, and angiotensin-converting enzyme (ACE) activity in 31 adult patients undergoing elective coronary artery bypass grafting, just before and up to three days after surgery, and in 25 healthy volunteers. **Results:** Patients showed higher plasma sP-s and sE-s and ACE concentrations, just before surgery, but significantly lower TN levels, compared with controls. During

the first three postoperative days (PD), the concentration of each of the molecules followed a different and independent pattern, although in the third PD, the levels of sP-s, sE-s and ACE were higher and those of vWF and TN lower, compared with the preoperative ones. However, patients had higher sP-s ($P = 0.06$), sE-s ($P = 0.07$), and vWF ($P = 0.005$), but lower TN concentrations ($P = 0.02$) on the third PD compared with controls. **Conclusions:** CPB is characterised by pronounced changes in plasma sP-s, sE-s, TN, vWF levels, and ACE activity, which are associated with significant alteration in the intra- and early postoperative endothelial function observed in open heart surgery. *J. Clin. Lab. Anal.* 24:389–398, 2010. © 2010 Wiley-Liss, Inc.

Key words: angiotensin-converting enzyme; cardiopulmonary bypass; E-selectin; P-selectin; tetranectin; von Willebrand factor

INTRODUCTION

In patients undergoing open heart surgery by means of cardiopulmonary bypass (CPB)-activated endothelium manifests a two-stage systemic inflammatory response syndrome (SIRS) accompanied by the compensatory responses of the organism to regain homeostasis (1,2). Complex mechanisms involving humoral and cellular immune pathways as well as altered flow state (increased shear stress) and hypothermia contribute to the activation and expression of complement, adhesion molecules, the synthesis of proinflammatory, prothrombotic factors, and the abnormal modulation of vascular tone (3). In the short-term stage of SIRS, which is lasting seconds to minutes, reactive oxygen species and activated complement fragments induce the transient expression of preformed proteins by endothelial cells, promoting leukocyte–endothelial cell interactions and coagulation. In the long-term stage, which occurs several hours later, a transcriptional activation of

several genes and their translation into proteins take place and regulate the recruitment of leukocytes and the formation of intravascular thrombin (4,5). The CPB-caused perturbation of the endothelium leads to the transformation of endothelial cells through programmatic biochemical changes from that of a resting condition to that expressing a prothrombotic surface (6). Homeostatic forces tend to achieve a new equilibrium between these two states, often permitting the injured endothelium to return to its unperturbed state once the

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procoagulant stimulus has dissipated, depending as well on perioperative stress responses and their impact on the cardiovascular system (7). There is a considerable heterogeneity in the degree to which different inflammatory pathways are activated after CPB, with genetic factors playing a crucial role (7).

CPB has generally been associated with increased levels of plasma soluble adhesion molecules of endothelial origin, which have been attributed to the activation and injury of endothelial cells. Thus, the availability of useful biochemical markers for evaluating the endothelial state postoperatively offers a useful non-invasive way to assess the endothelium's return to the unperurbed state, in order to improve patient outcomes.

Circulating soluble forms of P-selectin (sP-s) and E-selectin (sE-s), tetranectin (TN), von Willebrand factor (vWF), and angiotensin-converting enzyme (ACE) are plasma markers, which are exclusively or partially derived from endothelial cells and have linked to cardiovascular disease offering in the prognosis of cardiovascular patient. P-selectin is expressed constitutively in the α -granules of platelets, in Weibel-Palade bodies of endothelial cells, and on the surfaces of activated platelets and endothelial cells, whereas E-selectin is exclusively expressed by the cytokine-activated endothelial cells.

The endothelial cell-expressed P-selectin together with E-selectin mediate the initial step of weak and reversible leukocytes rolling along the vessel wall. They thus possess a central role in the transmigration of activated leukocytes in the subendothelial space, where they degranulate, promoting inflammatory injury. Elevated concentrations of sE-s and sP-s have been reported in a broad spectrum of pathologies ranging from hypercholesterolemia (8) and peripheral arterial occlusive disease to coronary artery disease (CAD) (9). High sP-s could not only reflect platelet or endothelial cell activation but also acts as a direct inducer of procoagulant activity associated with vascular and thrombotic diseases (10). TN, a homotrimeric adhesive molecule of the C-type superfamily of lectins, is also found in endothelial cells and platelets as well as in a mobilizable set of neutrophil granules, in monocytes, fibroblasts, and other cells (11). TN is released by platelets upon their activation by thrombin and probably participates in thrombus dissolution. Its biological role is probably based on its binding capacities; it binds to plasminogen kringle 4 domain and to kringle 1–4 domains of angiotensin (ASTK1–4). Thus, it enhances plasminogen activation by tissue-type plasminogen activator (12) and partially counteracts the ability of ASTK1–4 to inhibit the proliferation of endothelial cells (13).

vWF is an endothelial ligand for platelet glycoproteins, which is secreted by Weibel-Palade bodies after endothelial cell injury or by activated platelets. The role

of vWF in thrombus formation is to mediate the adhesion of platelets to the components of extracellular matrix and to one another and to protect factor VIII from proteolysis. Because of its biological function and the mode of its secretion by endothelial cells, vWF is considered the gold standard in the measurement of endothelial damage and is found to predict the risk of ischemic heart disease or stroke (14). ACE is a cell surface ectoenzyme, which hydrolyzes a number of substrates but its main known function is to cleave histidyl-leucine from angiotensin I to form angiotensin II extracellularly (15). Plasma ACE in healthy subjects arises essentially from the endothelial cells and it has recently been suggested that in high levels it may represent a risk factor for coronary stent restenosis, CAD, and myocardial infarction (16).

In this study, we measured plasma concentrations of sE-s, sP-s, TN, vWF, and ACE activity in adult patients undergoing elective coronary artery bypass grafting (CABG) surgery by means of CPB just before and up to 3 days after surgery. Another target of the study was to correlate these markers with patients' demographics and several perioperative characteristics as well as with the duration of surgery.

MATERIALS AND METHODS

Study Design-Setting

This is a prospective case-control study, which was conducted at the Onassis Cardiac Surgery Center, Athens, Greece that is a referral Center for patients with cardiovascular diseases. Approval was obtained from the Ethics and Research Committee of the Center. All patients and controls provided written informed consent.

Subjects

Thirty-one adult patients who underwent elective CABG surgery with CPB of mean age 63.3 years (range 54–74 years) were included in this study. All patients had stopped aspirin at least 7 days before surgery. No peripheral or carotid arterial disease or any other systemic disease was found by preoperative clinical and biochemical examination. Preoperative angiographic examinations of all had shown that complete revascularization was possible. Patients with a recent (<30 days) myocardial infarction or recent percutaneous transluminal coronary angioplasty and those with severe comorbidity were excluded from the study. Moreover, a control group was included in the study, composed of 25 volunteers (12 females, 13 males, mean age 64.8 ± 8.2 years) who did not undergo any cardiac operation. None of these subjects received medication for at least 2 weeks before the study.

Data Collection

Blood sampling

Blood samples (10 ml) were obtained from a peripheral venous line of patients just before the induction of general anesthesia and during the first and third post-operative day (PD) for measuring sP-s, sE-s, TN, vWF, and ACE activity. Complete blood counts were obtained using a Coulter HmX Haematology Analyzer (Coulter, Miami, FL). A portion of blood was anticoagulated with potassium EDTA (final concentration 5 mmol/l) to determine TN and the remainder with citrate (one volume of 0.11 mol/l citrate added to nine volumes of blood) to determine vWB, sP-s, sE-s, and ACE activity. Platelet-free plasma was obtained after centrifugation of the blood at $3,000 \times g$ for 10 min and 4°C and all plasma samples were stored at -80°C until analysis. The values of plasma soluble adhesion molecules, TN, vWF, and ACE activity were corrected by hemodilution, based on hematocrit values of each patient.

Laboratory assays—immunoassays

Plasma concentrations of sP-s were determined with a commercially available ELISA for sP-s (R&D systems, Minneapolis, MN), using monoclonal antibody specific for sP-s for microtiter plate preincubation and polyclonal antibody specific for sP-s conjugated to horseradish peroxidase. Inter- and intra assay coefficients of variation (CV) were $<8.8\%$ and $<5.2\%$, respectively, and sensitivity $<0.5\text{ ng/ml}$. Plasma concentrations of sE-s were determined with a commercially available ELISA for sE-s (R&D systems, Minneapolis, MN), using monoclonal antibody specific for sE-s for microtiter plate preincubation and polyclonal antibody specific for sE-s conjugated to horseradish peroxidase. Inter- and intra assay coefficients of variation (CV) were less than 8.2 and 4.8%, respectively, while the sensitivity of the assay was less than 0.1 ng/ml .

vWF was assessed by using a commercially available Elisa for vWF (Asserachrom® vWF; Diagnostica Stago, Asnieres, France), using microtiter plate having wells coated with rabbit anti-human vWF antibodies to capture the samples' vWF and with rabbit anti-vWF antibody coupled with peroxidase to bind the remaining free antigenic determinants of vWF to form the "sandwich." Plasma concentration of TN was assessed by using ELISA, using specific immunoglobulin against human TN and peroxidase-conjugated rabbit anti-human TN (Dakopatts Ltd, Denmark), as previously described (17). Serial dilutions of purified TN or pooled plasma were used for the construction of the standard curve. Inter- and intra assay coefficients of variation (CV) were $<7\%$ and $<4\%$, respectively.

ACE activity was determined using a colorimetric kit (Sigma Windham, NH). The assay was based on the ability of ACE enzyme to hydrolyze the synthetic substrate *N*-[(3-furyl) acryloyl]-L-phenyl-alanyl-glycyl-glycine and form furyl acryloyl phenyl-alanyl (FAP) and glycyl-glycine with a measurable decrease in optical density at a wavelength of 340 nm. One ACE activity unit was defined as 1 mmol of FAP produced per minute.

Management—Clinical Assessment

Anesthesia protocol

Anesthetic premedication protocol included lorazepam 2.5 mg orally the night before surgery and morphine sulfate (0.1–0.75 mg/kg) and promethazine (25–50 mg) both given intramuscularly 1 hr and 30 min, respectively, before the induction of general anesthesia. General anesthesia was then induced by intravenous administration of etomidate (0.2–0.3 mg/kg), midazolam (1–2 mg), fentanyl (10–15 $\mu\text{g/kg}$), and pancuronium or vecuronium (0.15 mg/kg). After the induction of general anesthesia, all patients underwent orotracheal intubation and anesthesia was maintained with additional doses of fentanyl up to a maximum total dose of $50\text{ }\mu\text{g/kg}$, isoflurane or sevoflurane. Additional doses of neuromuscular blocking agents were given when necessary. Cardiac output was measured by means a Swan-Ganz catheter with the thermodilution technique using a SC 9000 monitor (Siemens, Erlangen, Germany).

Intraoperative management and extracorporeal circulation

The patients were operated on with CPB at 33°C (esophageal temperature) using a hollow-fiber membrane oxygenator (Quadrox; Jostra, Hirrlingen, Germany) and an arterial filter. Heparin was given before cannulation to maintain an activated clotting time longer than 480 s during bypass. Bypass was conducted using pump flow rates in the range of 2.0–2.5/min per m^2 body surface area with arterial blood pressure maintenance at 50–75 mmHg. The aorta was cross-clamped 3–5 min after initiation of CPB. After the aorta was unclamped, the return of myocardial activity was recorded. If ventricular fibrillation (VF) appeared (reperfusion VF), a xylocaine 100 mg bolus was administered in the extracorporeal circuit. Internal defibrillation with 10–30 J was employed if VF persisted for more than 30 sec after the xylocaine bolus was administered. After discontinuation of extracorporeal circulation, protamine sulfate was given to neutralize heparin. Low cardiac index (less than $2.2\text{ L min}^{-1}\text{ m}^{-2}$) and hypotension (arterial systolic blood pressure $<100\text{ mm Hg}$)

persisting despite adequate volume administration were treated with intravenous infusion of inotropic agents.

Cardioplegia and controlled reperfusion protocol

Induced global myocardial ischemia and cardioprotection with hyperkalemic blood cardioplegia were used during the construction of distal coronary anastomoses. The cardioplegic solution was prepared using blood and a commercial cardioplegic solution (Cardioplegia Infusion; Martindale, Romford, UK) at 4°C, at a 4:1 ratio, in a standard blood cardioplegia setting (Avecor, Myotherm XP 4:1; Medtronic, Minneapolis, MN), and cold blood cardioplegia was administered retrograde through coronary sinus and antegrade through the ascending aorta, to all patients. The myocardial temperature was monitored with an 18-mm, 22-gauge Mon-a-therm myocardial temperature probe (Mallinckrodt Medical, St. Louis, MO) placed in the anterior interventricular septum. During aortic occlusion, myocardial temperature was kept between 10 and 18°C with repeated infusions of cardioplegic solution and topical ice slush solution. After completion of all distal coronary anastomoses and before removal of the aortic cross-clamp, all patients received controlled myocardial reperfusion in a retrograde fashion. The reperfusion perfusate (1 L) consists of 250 ml of warm blood cardioplegic solution ("hot shot") that was chased by 750 ml of pump blood ("chase").

Postoperative management

After surgery, the patients were transferred intubated to the intensive care unit where standard postoperative management was carried out. The electrocardiogram, the arterial and central venous pressures, and the mixed venous oxygen saturation were continuously monitored. Inotropic agents (dobutamine or epinephrine, or both) were infused intravenously when needed (systolic blood pressure <100 mm Hg not responding to volume administration, cardiac index <2.2 L min⁻¹ m⁻²). When necessary, vasodilators (nitroglycerin and/or nitroprusside) were administered intravenously to control arterial hypertension, xylocaine to control ventricular ectopy, and furosemide to enhance diuresis. Red blood cells were transfused to keep the hematocrit above 26%. Appearance of ventricular arrhythmias, low cardiac output, or any complication, including the need for inotropic or antiarrhythmic agents or for pacing, was recorded in all patients.

Study outcomes

The primary end point was the differences in plasma concentrations of endothelial origin biomarkers sE-s,

sP-s, TN, vWF, and ACE activity between adult patients undergoing elective CABG surgery by means of CPB and healthy subjects (controls) just before and up to 3 days after surgery. Secondary end points were the correlation of these markers with patients' demographics and several perioperative characteristics as well as with duration of CPB and aortic cross-clamp time (ACCT).

Statistical analysis

Data were analyzed by using the Statistical Package for the Social Sciences for Windows, version 10.0. All data were expressed as a mean with one standard deviation and were graphically presented as bar charts. Statistical analysis was performed with the mixed model two-way repeated ANOVA to evaluate the interaction of time with each variable separately. The same model was used, when needed, in order to elucidate the specific differences of variables between different sampling points. For the analysis of variance over time, the separate one factor repeated measures ANOVA were performed, when needed, in order to compare specific variables between each sampling point. Continuous data were summarized as mean ($\pm$ SD) while categorical data were represented as number (proportions). Pearson's Chi square test was used to compare categorical data. Mann-Whitney *U*-test was used to analyze the endothelial marker levels between control and patient groups as well as of the percentage change of each variable from the baseline value between groups differing in CPB time (CPBT) and ACCT, as some parameters did not appear to be normally distributed. A two-tailed *P*-value less than 0.05 was considered statistically significant.

RESULTS

Subjects

Thirty-one patients who underwent aortocoronary bypass surgery with CPB were included in this study. All had stable symptoms with NYHA class I–IV functional capacities. Demographics and clinical characteristics of all patients and controls enrolled to the study are presented in Table 1. All patients received an internal mammary artery graft. The mean number of grafts was 3 ± 1 (range 1–5). Mean ACCTs and CPBTs were 65.8 ± 17.7 min and 103.9 ± 27.3 min, respectively. Intra-operatively, seven patients (22.6%) needed electrical defibrillation and three patients (9.7%) needed pacemaker. All patients were discharged from the ICU and from the hospital within 1 and 7 days after surgery, respectively. In hospital, mortality was zero.

TABLE 1. Baseline Characteristics

Characteristics	Patients (n = 31)	Controls (n = 25)	P-value
Age (years)	63.3 ± 7.8	63.0 ± 5.6	0.87
Sex (n (%))			
Male	23 (74.2)	19 (76.0)	0.87
Female	8 (25.8)	6 (24.0)	
Smoking (n (%))	19 (61.3)	16 (64.0)	0.94
Arterial hypertension (n (%))	16 (51.6)	10 (40.0)	0.55
Hyperlipidemia (n (%))	21 (67.7)	16 (64.0)	0.99
AMI (n (%))	18 (58.1)	4 (16.0)	0.003
LVEF (%)	0.47 ± 0.09		
Diabetes mellitus (n (%))	5 (16.1)	3 (12.0)	0.95
COPD (n (%))	1 (3.2)	1 (4.0)	0.56

Results are n (%) or mean ± SD. AMI, acute myocardial infarction; COPD, chronic obstructive pulmonary disease; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

TABLE 2. Baseline Biomarker Levels

Biomarker	Patients (n = 31)	Controls (n = 25)	P-value
Soluble P-selectin (ng/ml)	69.05 ± 12.06	29.49 ± 9.32	0.04
Soluble E-selectin (ng/ml)	55.05 ± 4.03	35.27 ± 2.23	0.05
Tetranectin (mg/l)	6.42 ± 0.34	12.28 ± 0.27	0.001
von Willebrand factor (%)	94.19 ± 6.37	75.12 ± 4.25	0.47
Angiotensin-converting enzyme (U/l)	43.55 ± 3.75	31.52 ± 2.11	0.01

Results are expressed as mean ± SD.

A statistically significant difference was found between patients and controls regarding preoperative values of plasma concentrations of sP-s (69.05 ± 12.06 ng/ml vs. 29.49 ± 9.32 ng/ml, $P = 0.04$), sE-s (55.05 ± 4.03 ng/ml vs. 35.27 ± 2.23 , $P = 0.05$), TN (6.42 ± 0.34 mg/l vs. 12.28 ± 0.27 mg/l, $P = 0.001$), and ACE activity (43.55 ± 3.75 U/l vs. 31.52 ± 2.11 U/l, $P = 0.01$). On the contrary, no statistically significant difference was found between patients and controls in preoperative plasma levels of vWF ($94.19 \pm 6.37\%$ vs. $75.12 \pm 4.25\%$, $P = 0.47$) (Table 2).

No statistically significant differences were found in plasma values for any of the examined endothelial markers between men and women, patients with or without pre-existing arterial hypertension, hypercholesterolemia or AMI, and patients aged older than 61 or younger than 60 years. On the contrary, there was a statistically significant difference in plasma TN levels (7.29 ± 2.43 , 5.95 ± 1.17 mg/l, $P = 0.04$) between patients with NYHA class I–II and those with NYHA class III–IV.

For patients, the changes of sP-s, sE-s, vWF, TN levels, and ACE activity within time intervals are illustrated in Figures 1–5. The statistical analysis (two-way repeated ANOVA) revealed a significant effect of

Fig. 1. Plasma levels of sP-s in 30 patients undergoing CPB. Time 0, before CPB; time 1, the first postoperative day; time 3, the third postoperative day. Results are expressed as mean ± SD. * $P = 0.048$ vs. preoperative sP-s. sP-s, soluble P-selectin; CPB, cardiopulmonary bypass.

Fig. 2. Plasma levels of sE-s values in 30 patients undergoing CPB. Time 0, before CPB; time 1, the first postoperative day; time 3, the third postoperative day. Results are expressed as mean ± SD. * $P < 0.05$ vs. preoperative and first postoperative sE-s values. sE-s, soluble E-selectin; CPB, cardiopulmonary bypass.

time for each of the examined variables (sP-s, $P = 0.05$, sE-s, $P < 0.05$, vWF, $P < 0.0005$, TN, $P = 0.01$, and ACE activity, $P = 0.01$). In particular, sP-s showed a tendency toward an increase in plasma levels during the first PD, but on the third PD it decreased to levels lower than baseline and those of the first day ($P = 0.04$) (Fig. 1). The time profile of sE-s alteration differed

Fig. 3. Plasma levels of vWF in 30 patients undergoing CPB. Time 0, before CPB; Time 1, the first postoperative day; Time 3, the third postoperative day. Results are expressed as mean $\pm$ SD. * $P < 0.0005$ vs. others. vWF, von Willebrand factor; CPB, cardiopulmonary bypass.

Fig. 4. Plasma TN levels in 30 patients undergoing CPB. Time 0, before CPB; Time 1, the first postoperative day; Time 3, the third postoperative day. Results are expressed as mean $\pm$ SD. * $P = 0.04$ vs. preoperative tetranectin values; ** $P = 0.05$ vs. first postoperative TN values. TN, tetranectin; CPB, cardiopulmonary bypass.

from that of sP-s, as sE-s levels showed a gradual decrease. They were significantly lower during the third PD compared with baseline ($P < 0.05$) or with those of the first day ($P < 0.05$) (Fig. 2). The plasma levels of vWF, however, had risen on the first PD, reaching their peak levels, while during the third PD they showed a significant decrease but still remained elevated compared with the baseline values ($P < 0.0005$) (Fig. 3). On

Fig. 5. Plasma ACE activity in 30 patients undergoing CPB. Time 0, before CPB; Time 1, the first postoperative day; Time 3, the third postoperative day. Results are expressed as mean $\pm$ SD. * $P = 0.01$ vs. preoperative ACE activity. ACE, angiotensin-converting enzyme; CPB, cardiopulmonary bypass.

the other hand, TN plasma levels, interestingly, showed a gradual elevation on the first ($P < 0.04$) and third ($P = 0.05$) PD (Fig. 4). Soluble ACE activity, however, showed a gradual decrease, with its activity to be lower compared with the baseline, however, not reaching the point of statistical significance on the first day, but only on the third PD ($P = 0.01$) (Fig. 5).

At the end of the observation period (third PD), there was no significant difference in sP-s (53.8 ± 30.7 ng/ml vs. 29.5 ± 11.3 ng/ml, $P = 0.06$), sE-s (41.2 ± 18.2 ng/ml vs. 35.3 ± 2.4 ng/ml, $P = 0.07$), and ACE activity (33.8 ± 4.1 U/l vs. $31.5 \pm 31.5 \pm 4.1$ U/l, $P = 0.99$) between groups (patients vs. controls). On the contrary, patients had increased levels of vWF ($179.0 \pm 87.3\%$ vs. $75.1 \pm 17.2\%$, $P < 0.005$), but significantly lower plasma TN levels (8.0 ± 0.38 mg/l vs. 12.3 ± 1.0 mg/ml, $P = 0.02$) compared with controls.

To identify the effect of CPB and aortic cross-clamp duration on the biomarkers examined, patients were divided based on CPBT into two groups: those with less or more than 100 min. This analysis showed that there was no statistical difference in sP-s ($P = 0.10$), sE-s ($P = 0.14$), vWF ($P = 0.40$), and ACE activity ($P = 0.11$) between these two groups. On the contrary, TN presented significant interaction with CPBT ($P = 0.04$), indicating that the duration of CPB influences the profile of TN fluctuation in time. As far as ACCT, patients were divided in those with ACCT above 61 min and those with ACCT less than 60 min. Similarly, the analysis showed that there was no statistical interaction of sP-s ($P = 0.15$), sE-s ($P = 0.18$), vWF ($P = 0.40$), and

ACE activity ($P = 0.58$) with the ACCT, demonstrating that these biochemical factors followed the same pattern of fluctuation in time independently of aortic cross-clamp duration. On the contrary, TN presented a statistically significant interaction with ACCT and sampling time ($P = 0.04$), indicating that TN presented a different profile of alteration in time in patients differing in ACCT. Specifically, patients with ACCT <60 min presented higher plasma TN than those with ACCT >60 min.

In addition, we estimated the percentage change of each endothelial marker on the first and third PD from their baseline levels. The statistical analysis of the resulting percentage disparities then showed no significant difference in the percentage change of sE-s, vWF, and ACE, on the first and third day from baseline levels of patients differing in CPBT or ACCT. However, the percentage differences from baseline levels of sP-s and TN between patients differing in CPBT and ACCT, though not statistically significant on the first PD, they were statistically significant on the third PD (Figs. 6 and 7).

DISCUSSION

The main finding of this study was that increased plasma values were found in the majority of endothelial origin biomarkers, such as sP-s, sE-s, and ACE activity in patients undergoing CABG surgery with CPB, compared with healthy controls. On the contrary, patients had decreased plasma TN levels compared with controls. We also found that CPB induces biphasic changes in sP-s and vWF, peaked at high levels on the first PD and diminished on the third PD. In addition, CPB induces a gradual decrease in sE-s and ACE activity as well as a gradual increase in TN levels. Consequently, after CPB, an additional inflammatory response developed, inducing the activation of various inflammatory pathways. Thus, preformed molecules like P-selectin, vWF, and TN, which are stored in the granules of endothelial cells and platelets could be released due to the contact with CPB circuit.

First, we compared the selected endothelial origin biomarkers between control subjects (NYHA class I) and patients undergoing CABG with CPB, given that all the patients had stable symptoms with NYHA class I–IV functional capacities and well-defined CAD the severity of which was shown by preoperative angiographic examinations with cardiac catheterization. The significant differences in the preoperative plasma sP-s, sE-s, and TN levels as well as in the ACE activity found was probably due to differences in the degree of the pre-existed endothelial inflammation or dysfunction and the severity of atherosclerotic lesions between these groups.

Our findings concerning patients undergoing CABG surgery with CPB are consistent with those of previous

Fig. 6. Percentage difference of tetranectin (top) and sP-selectin (bottom) plasma values from baseline values (preoperative values) of patients undergoing cardiopulmonary bypass with CPB duration <100 min (closed circles), and those with CPB duration ≥100 min (open circles). Differences between groups are significant at the 0.05 level.

studies and are indicative of the endothelial inflammation/damage combined with the demonstrated coronary disease of the patients selected for CPB (18–20). However, in this study, plasma concentrations of five different biomarkers of endothelial origin were measured in adult patients undergoing on-pump coronary artery revascularization with hypothermic cardiac arrest three times; just before surgery, on the first and the third PD in order to examine endothelial injury and/or activation by means of CPB circuit in these patients. We found that CPB was associated with pronounced changes in plasma concentrations of sP-s, sE-s, TN, vWF, and ACE activity in adult patients undergoing CABG surgery.

The endothelial cell-expressed P-selectin together with E-selectin mediate the initial step of weak and reversible leukocytes rolling along the vessel wall. Thus, they possess a central role in the transmigration of

Fig. 7. Percentage difference of tetranectin (top) and sP-selectin (bottom) plasma values from baseline values (preoperative values) of patients undergoing cardiopulmonary bypass with aortocoronary cross clamp duration (ACCT) <60 min (closed circles), and those with ACCT >61 min (open circles). Differences between groups are significant at the 0.05 level.

activated leukocytes in the subendothelial space, they degranulate, promoting inflammatory injury. Elevated concentrations of sE-s and sP-s have been reported in a broad spectrum of pathologies ranging from hypercholesterolemia (8) and peripheral arterial occlusive disease to CAD (9).

High sP-s could not only reflect platelet or endothelial cell activation but also acts as a direct inducer of procoagulant activity associated with vascular and thrombotic diseases (21). Plasma sP-s has been shown to predict major cardiovascular events in patients with existing peripheral or coronary atherosclerosis and even in apparently healthy women (21). However, a previous study found a decrease in sE-s during CPB and an increase in its levels at 48 h after CPB (22).

vWF is secreted by Weibel-Palade bodies after endothelial cell injury or activated platelets. The vWF plays a role in platelet adhesion and aggregation, and

protects factor VIII from proteolysis. Because of its biological function and the mode of its secretion by endothelial cells, vWF could be used as a highly relevant biomarker for endothelial dysfunction/damage as well as the thrombotic potential of the organism (14). In addition, vWF was found to predict ischemic heart disease in healthy individuals and in patients with angina pectoris or acute myocardial infarction. vWF levels appear to be an independent predictor of subsequent acute myocardial infarction or mortality (23).

TN, a homotrimeric adhesive molecule of the C-type superfamily of lectins, is also found in endothelial cells and platelets as well as in a mobilizable set of neutrophil granules, in monocytes, fibroblasts, and other cells (11). TN is released by platelets on their activation by thrombin and probably participates in thrombus dissolution. Its biological role is probably based on its binding capacities; it binds to plasminogen kringle 4 domain and to ASTK1-4, enhancing plasminogen activation by tissue-type plasminogen activator and partially counteracting the ability of ASTK1-4 to inhibit the proliferation of endothelial cells (13). Decreased plasma TN levels were found in patients with CAD with gradually lower values from stable to unstable angina until acute myocardial infarction, and therapeutic treatment with rTPA has resulted in an increase of plasma TN levels (24,25). The difference of TN response to CPB postoperatively compared with the biphasic alteration of sP-s could be due to the fact that plasma TN levels are strictly regulated depending on TN release by cells, TN bound in fibrin or other molecules, and TN excretion from the body. Thus, with the end of CPB, the observed TN increase could be due to TN gene upregulation and/or to the fact that there is less fibrin formed and thus less TN trapped in it. This results in even higher plasma TN levels.

The renin-angiotensin system and ACE activity has been found to be heavily involved in the inflammatory vascular disease. ACE is a cell surface ectoenzyme whose main known function is to cleave histidyl-leucine from angiotensin I to form angiotensin II extracellularly (15). Plasma ACE in healthy subjects arises essentially from the endothelial cells and it has recently been suggested that in high levels it may represent a risk factor for coronary stent restenosis, CAD, and myocardial infarction (16). Similarly, the activity of ACE is enhanced in coronary samples of patients with unstable angina (26) and appears to represent a risk factor for CAD.

It was shown by previous studies that short-term hypoxia or anoxia also induces increased adhesion of leukocytes to vascular endothelial cells mediated by the upregulation of endothelial adhesion molecules (27). Moreover, cardiac surgery with CPB induces ischemia in the heart and hypoxemia in various tissues as well as a

surge of endotoxins and cytokines (4). Additionally, genes of TN and CD24, a P-selectin ligand that can be expressed by activated vascular endothelium and platelets, are found to be strongly upregulated in HUVEC exposed to hypoxia (28). It could be assumed that the increase in the levels of sP-s, sE-s, vWF, TN and in ACE activity on the first PD mirrored endothelial injury due to CPB process inflammation. Specifically, for the TN alteration profile, this increase could be due to a hypoxia-induced gene upregulation.

The drop in vWF and sP-s levels on the third PD in conjunction with a further increase in TN levels reflects an alteration in the balance between coagulation/fibrinolysis in favor of fibrinolysis, as TN is considered to be a fibrinolytic regulator involved in plasmin cascade. The significant difference is not only of sP-s and TN values but also of sE-s and ACE activity on the third PD from baseline patient values, and their tendency to return to those of healthy volunteers, enhances the hypothesis of induction of compensatory responses of the organism to regain homeostasis. The absence of stimulatory inputs, which activate inflammatory pathways leading to pro-coagulant activity, adhesion molecules upregulation, and rennin-angiotensin system activation lead to the less endothelial injury, resulting in the decrease of procoagulant proinflammatory molecules.

Based on our results, prolonged duration of both CPBT and ACCT, which are related to the degree of hypoxia on endothelial dysfunction, significantly affects the percentage difference of sP-s and TN from baseline levels, especially on the third PD. Our findings concerning the impact of CPB and ACC duration on endothelial markers as well as the percentage change in their levels from baseline levels between patients differing in CPB and ACC duration showed that from the examined endothelial markers only sP-s and TN were influenced, especially on third PD. Patients with CPBT less than 100 min or ACCT less than 60 min presented lower sP-s and higher TN levels than those with longer CPBT and aortic cross-clamp duration. It is tempting to speculate that on the third PD the compensatory mechanisms activated in patients work more efficiently in those with shorter CPBT and ACCT and perhaps with a lesser degree of dysfunction/injury of the endothelium. The balance of sP-s and TN plasma levels seems to provide a good indication of patient recovery after cardiac surgery with CPB. However, this needs to be confirmed in larger studies.

We conclude that CPB with hypothermic cardiac arrest induces a transient and moderate endothelial injury and dysfunction. The increase in the fibrinolytic regulator TN represents an endothelial attempt in order to be restored homeostasis between coagulation and fibrinolysis early postoperatively in this group of patients. The continuously elevated TN levels in conjunction with the

significant lowering of vWF, P-selectin, E-selectins, and ACE activity observed on the third PD are indicative of a tendency for the endothelium to regain its protective role. The above alterations could be related with the development of SIRS, observed in open heart surgery. These biomarkers could be a useful tool in assessing endothelial status in early postoperative period in adult patients undergoing on-pump cardiac surgery.

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