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The role of tissue factor and P-selectin in the procoagulant response that occurs in the first month after on-pump and off-pump coronary artery bypass grafting

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Supplemental material is available online.

Background: It has been previously shown that a persistent (up to 1 month) prothrombotic status occurs after coronary bypass surgery performed both on pump and off pump. To assess the pathways involved in the occurrence of postoperative prothrombotic state, in this study we evaluated plasma, monocyte-bound, and platelet-bound tissue factor expression, as well as platelet and soluble P-selectin expression, up to 1 month after off-pump and on-pump coronary artery bypass grafting.

Methods: Thirty patient candidates for coronary surgery were randomized to undergo off-pump coronary artery bypass grafting (n = 15) or on-pump coronary artery bypass grafting (n = 15). Blood samples were collected before the intervention, after protamine administration, and 4, 8, and 30 days after surgical intervention.

Results: Plasma tissue factor levels were significantly higher than baseline both in the on-pump coronary artery bypass grafting group (from protamine administration up to 4 postoperative days) and in the off-pump coronary artery bypass grafting group (at 4 postoperative days), with no differences between groups. Basal and lipopolysaccharide-stimulated monocyte tissue factor expression, as well as basal and adenosine diphosphate–stimulated platelet tissue factor expression, did not show significant variations over time and were similar in the on-pump and off-pump coronary artery bypass grafting groups throughout the course of the study. Platelet expression of P-selectin, both basal and after adenosine diphosphate stimulation, did not significantly change over time and was not different in the on-pump and off-pump coronary artery bypass grafting groups. Soluble P-selectin levels in plasma were significantly higher in patients receiving on-pump coronary artery bypass grafting only at the time point after protamine administration, whereas this variable behaved similarly in the on-pump and off-pump coronary artery bypass grafting groups for the whole postoperative period.

Conclusions: The postoperative tissue factor and P-selectin expression did not differ between the on-pump and off-pump coronary artery bypass grafting groups. The distinct increase of plasma tissue factor occurring after both surgical procedures might represent a mechanism that might explain, in part, the early postoperative prothrombotic state occurring after on-pump and off-pump coronary artery bypass grafting.

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It has been previously shown that a sensible activation of the hemostatic-thrombotic and inflammatory systems occurs during the intervention and persists for several weeks after coronary artery bypass surgery.[1-4] This activation is more marked in patients undergoing on-pump coronary artery bypass grafting (CABG), which lasts for the limited time span during the operation and in the very

Abbreviations and Acronyms

ADP = adenosine diphosphate
ANOVA = analysis of variance
CABG = on-pump coronary artery bypass grafting
FITC = fluorescein isothiocyanate
GLM = general linear model
mAb = monoclonal antibody
MFI = mean fluorescence intensity
OPCAB = off-pump coronary artery bypass grafting
PE = phycoerythrin
TF = tissue factor
WB = whole blood

early hours thereafter, compared with that seen in patients submitted to off-pump coronary artery bypass grafting (OPCAB). At later times (eg, after the first postoperative hours up to 1 month after the operation), the degree of this activation is similar in patients undergoing both CABG and OPCAB.[5]

Both tissue factor (TF) pathways[6-8] and platelet activation[9] have been previously documented as mechanisms underlying the early hemostatic activation phase occurring during and in the early hours after coronary surgery. It is unclear, however, which pathway is involved in the occurrence and development of the late phases of this prothrombotic state that, unlike the early activation phase, seems to be unrelated to extracorporeal circulation use.[5]

In this study we have investigated whether TF and P-selectin play a role in the delayed activation of coagulation occurring after coronary surgery.

Patients and Methods

Patients

Thirty patient candidates for elective surgical myocardial revascularization according to the American Heart Association/American College of Cardiology guidelines[10] and in whom both OPCAB and CABG were considered feasible were enrolled during the time period from July 2003 through December 2003 and randomized to undergo OPCAB (n = 15) or CABG (n = 15). In all cases the preoperative ejection fraction was greater than 30%, and the left ventricular end-diastolic pressure was less than 20 mm Hg. Preoperative exclusion criteria were age of 80 years or older, renal or liver disease, intake of drugs affecting platelet function or coagulation, or fibrinolysis within 10 days before the operation, whereas intraoperative and postoperative exclusion criteria were excessive (>1000 mL/24 hours) postoperative bleeding or re-exploration for bleeding, perioperative myocardial infarction, stroke, or renal failure requiring dialysis. Clinical variables of patients undergoing CABG and OPCAB are reported in Table E1 (electronic pages).

All patients provided informed consent to participate in this study, which was approved by the Institutional Review Board of Centro Cardiologico Monzino IRCCS. All patients were managed by the same surgical and anesthesiologic team.

Anesthesia

Patient management during and after the operation was the same in both groups of patients. All patients continued their cardiac medications until the operation.

Patients received thiopentone, 3 to 5 mg/kg, and fentanyl, 1 μg/kg, as induction and were maintained with sufentanil boluses of up to 4 to 5 μg/kg associated with propofol (Diprivan, AstraZeneca) continuous infusion at 3 $mg \cdot kg^{-1} \cdot h^{-1}$.

After orotracheal intubation, patients were ventilated with oxygen and air (fraction of inspired oxygen, 50%), maintaining $Paco_2$ at between 35 and 38 mm Hg. Rectal and cervical esophageal probes were used for temperature monitoring, and acid-base equilibrium was maintained by using the alpha-stat method.

After internal thoracic artery takedown, systemic heparinization (300 IU/kg bovine lung heparin) was given in both groups, and anticoagulation was assessed with celite ACT, with a trigger level for additional heparin set at 440 seconds every 30 minutes during cardiopulmonary bypass (CPB; in the CABG group) or during coronary anastomosis confection (in the OPCAB group).

On completion of distal and proximal coronary anastomoses, heparin was antagonized with protamine sulfate at a 1:1 ratio (3 mg/kg) in both groups. The protamine dose was based on total heparin used during the operation.

CABG Surgery

A nonpulsatile roller pump, hollow-fiber oxygenator with integrated heat exchanger, arterial filter, open cardiotomy reservoir, and polyvinyl tubing system were used in all cases. Each operation was performed with tepid hypothermia (32°C-34°C) and hemodilution. Blood flow during CPB was kept at 2.4 $L \cdot min^{-1} \cdot m^{-2}$, and hematocrit was kept at 18% to 25%. Myocardial protection was achieved through the administration of cold (4°C) multidose blood cardioplegia infused through the aortic root and the coronary sinus.

OPCAB Surgery

All OPCAB surgeries were performed through a midline sternotomy; mechanical stability of the coronary arteriotomy area was achieved with a suction stabilizer, and a soft plastic coronary flow shunt was always introduced into the coronary arteriotomy to maintain some degree of distal flow, to reduce myocardial ischemia, and to improve visualization of the anastomosis area. Coronary artery exposure was achieved with stay sutures applied on the left lateral side of the pericardium or with deep pericardial stay sutures placed above the entry to the left lower pulmonary vein and laterally to the entry of the inferior vena cava (Lima stitch).

Follow-up

All the patients were hospitalized until the 8th postoperative day. Then all patients underwent a follow-up visit (physical examination, electrocardiograph, and blood collection) at the 30th postoperative day.

Blood Sampling

Blood collection was performed from the antecubital vein through a 19-gauge needle without venous stasis. After discarding the first 4 mL, blood was drawn into citrate-containing (1:10 vol sodium citrate 0.129 mol/L) Vacutainer tubes (Becton Dickinson) at base-

line (the day before the operation), 5 minutes after protamine administration, at stable hemodynamic conditions, and at 4, 8, and 30 days after surgical intervention. Plasma was prepared by means of centrifugation at 1500*g* for 20 minutes at 4°C within 30 minutes from venipuncture, divided into aliquots, and frozen at −80°C until assayed.

For laboratory methods please see the Electronic Appendix.

Statistical Analysis

The study was powered to detect, with a power of 80%, an α error of .05, a percentage change from baseline equal to 1 standard deviation in any time point. Continuous variables are presented as means ± 1 standard error of the mean, and categoric variables are presented as percentages. Group differences in clinical variables between the CABG and OPCAB groups were assessed with analysis of variance (ANOVA) and χ^2 or Fisher exact tests when indicated.

General linear model (GLM) ANOVA models were used for statistical analysis of time, group (CABG vs OPCAB), and interaction (time * group) effects (main effects) in hematologic variables assessed as percentage variations from baseline values. When time, group, or interaction effects were significant ($P \leq .05$), repeated-measures ANOVA with the Bonferroni correction was used to determine significant ($P \leq .05$) point-by-point differences.

Results

Absolute values of tissue factor and p-selection are reported in the electronic pages (Table E2).

Tissue Factor

Plasma TF. Baseline plasma TF levels were similar in the CABG and OPCAB groups (157 ± 5.5 and 145 ± 6.2 pg/mL, respectively). GLM ANOVA showed a significant effect of time ($P = .045$) but not of treatment ($P = .57$); point-by-point analysis documented significant increases with respect to the baseline occurring both in the CABG group (after protamine: +72% ± 20%, $P = .02$; 4 days postoperatively: +83% ± 26%, $P = .03$) and the OPCAB group (4 days postoperatively: +98% ± 33%, $P = .05$; Figure 1, *A*).

Monocyte TF expression. Basal and lipopolysaccharide-stimulated monocyte TF expression was similar in the CABG and OPCAB groups before the operation (basal: 0.95 ± 0.043 and 0.95 ± 0.037 mean fluorescence intensity [MFI], respectively; stimulated: 1.59 ± 0.094 and 1.46 ± 0.115 MFI, respectively) and did not change in either group throughout the course of the study (Figure 1, *B* and *C*).

Platelet TF expression. Unstimulated and stimulated platelet TF expression was similar in the CABG and OPCAB groups before the operation (unstimulated: 2.15 ± 0.208 and 1.83 ± 0.150 MFI, respectively; stimulated: 3.37 ± 0.182 and 3.15 ± 0.230 MFI, respectively). The analysis of TF expression in unstimulated platelets showed a significant effect of time ($P = .018$); point-by-point analysis, however, did not show any significant difference with respect to baseline in both groups (Figure 1, *D*). Adenosine diphosphate (ADP)–induced platelet TF expression did not change throughout the study in either the CABG or OPCAB group (Figure 1, *E*).

P-selectin

Platelet P-selectin expression. Unstimulated and stimulated platelet P-selectin expression was similar in the CABG and OPCAB groups before the operation (unstimulated: 0.94 ± 0.019 and 0.90 ± 0.035 MFI, respectively; stimulated: 3.97 ± 0.496 and 2.67 ± 0.416 MFI, respectively). Both unstimulated and stimulated P-selectin expression did not show significant variations over time or differences between patients assigned to the CABG and OPCAB groups (Figure 2, *A* and *B*).

Soluble P-selectin. Soluble P-selectin levels were similar at baseline in the CABG and OPCAB groups (101 ± 3.7 and 93 ± 3.0 ng/mL, respectively). GLM ANOVA showed a significant effect of time ($P < .001$), treatment ($P < .036$), and interaction ($P = .013$) terms; point-by-point analysis showed no differences over time in the OPCAB group, whereas in the CABG group there was a significant increase with respect to baseline after protamine administration (+41% ± 10%, $P = .05$). At this time point, there was also a significant difference between the CABG and OPCAB groups (+41% ± 10% and −23% ± 10%, respectively; $P < .001$), whereas no differences could be demonstrated in the postoperative period both with respect to baseline and between the CABG and OPCAB groups (Figure 2, *C*).

Discussion

Several studies have documented major changes in hemostatic variables occurring during and early after CABG performed with the use of CPB. Activation of the extrinsic coagulation pathway occurs during surgical intervention and is due to blood that first comes into contact with nonendothelial surfaces of the pump and especially of the surgical wound and then is directed to the pump; this is an important trigger for intraoperative coagulation activation because it is followed by early and sharp increases of thrombin generation markers.[7,8] Likewise, sensible platelet activation has been documented to occur during and in the early phases after coronary surgery, being characterized by increased platelet expression of P-selectin and also increased levels of its soluble form.[9]

The above-mentioned evidence comes, however, from studies that were focused on the very early phase after surgical intervention, extending observation times for a maximum of 24 to 48 hours after CABG. A protracted prothrombotic state occurring after CABG was recently demonstrated,[1] even though the mechanisms underlying this state have not been investigated yet. Moreover, a sensible activation of hemo-

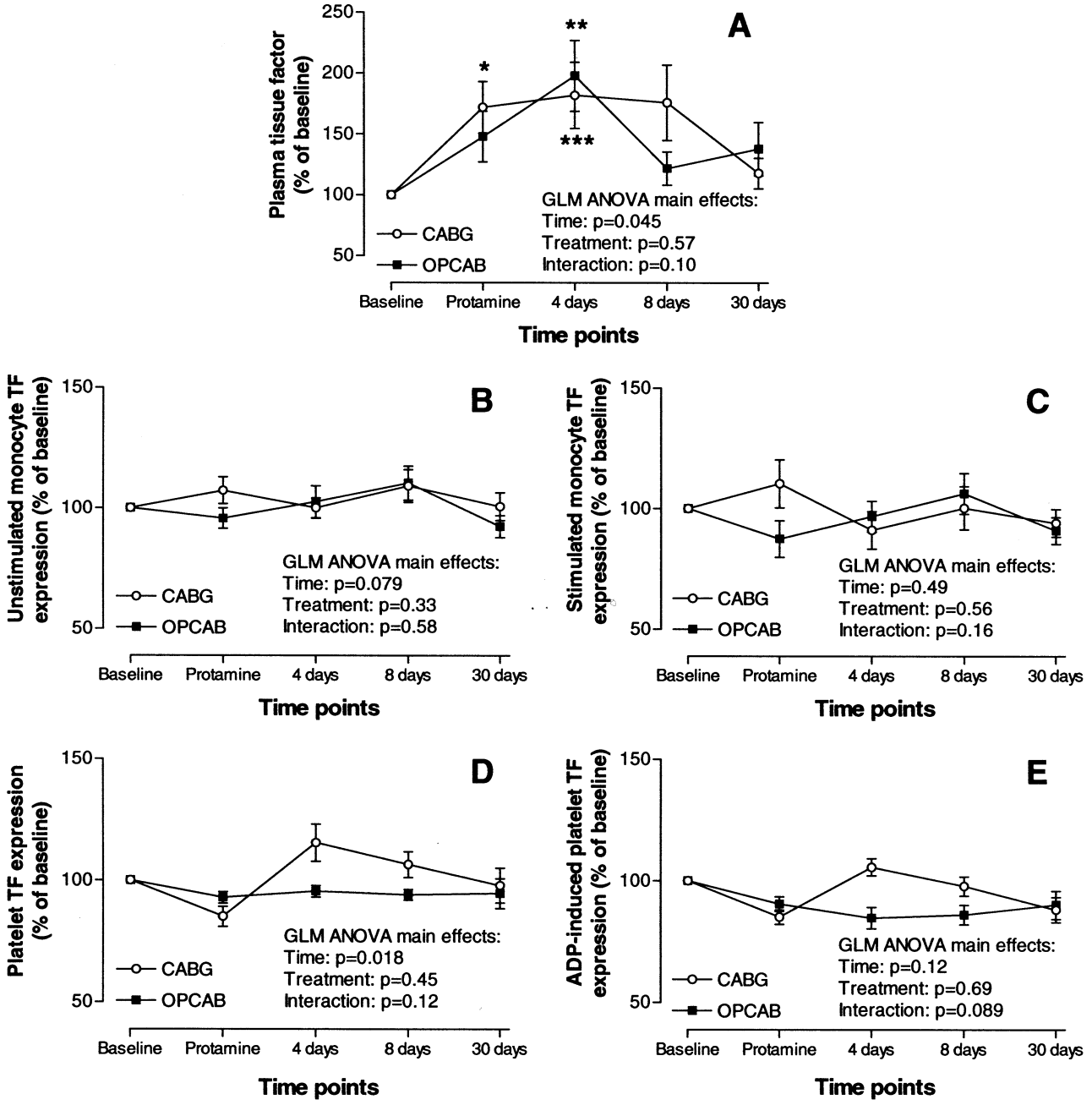

Figure 1. Tissue factor *(TF)*. Plasma tissue factor (A), baseline monocyte tissue factor (B), lipopolysaccharide-stimulated monocyte tissue factor (C), baseline platelet tissue factor (D), and adenosine diphosphate *(ADP)*–stimulated platelet tissue factor (E) are shown at each time interval. Data are presented as means ± 1 standard error of the mean. *Open circles* indicate patients assigned to on-pump coronary artery bypass grafting (*CABG*; n = 15), and *filled squares* indicate patients assigned to off-pump coronary artery bypass grafting (*OPCAB*; n = 15). **P* = .02, ***P* = .05, ****P* = .03 versus baseline. *GLM ANOVA*, General linear model analysis of variance.

static pathways has been documented also after coronary surgery performed off pump; this activation is of relatively lower degree in off-pump coronary surgery during and in the very early hours thereafter,[5,11] whereas at later times, the extent of this activation is similar between on-pump and off-pump procedures[5]; this led to the hypothesis that extra-

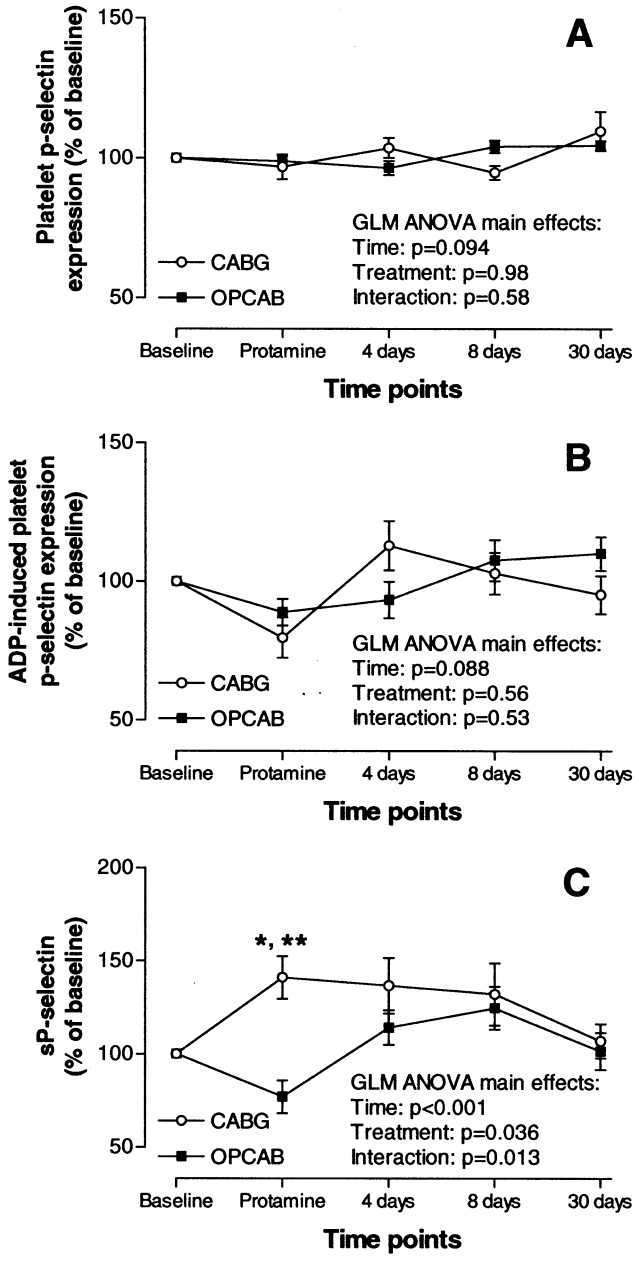

Figure 2. P-selectin. Baseline platelet P-selectin (A), adenosine diphosphate *(ADP)*–stimulated platelet P-selectin (B), and soluble P-selectin (C) expression are shown at each time interval. Data are presented as means ± 1 standard error of the mean. Symbols are as in Figure 1. *$P = .002$ vs baseline and **$P < .001$, on-pump coronary artery bypass grafting *(CABG)* versus off-pump coronary artery bypass grafting *(OPCAB)*. *GLM ANOVA*, General linear model analysis of variance.

corporeal circulation is the cause of the perturbations occurring early after surgical intervention, whereas surgical trauma is likely to be responsible for the later ones.[12]

Our study shows that the delayed activation of the coagulation that occurs after coronary bypass surgery is not caused by platelet activation. Indeed, established markers of platelet activation, platelet-bound P-selectin expression,[7] soluble P-selectin,[9] and a possibly new indicator of platelet activation, platelet-bound TF,[13] did not show sensible changes in the postoperative period up to 1 month.

In addition, the parallel in vivo behavior of basal and ADP-stimulated platelet expression of P-selectin, which not only mediates the adherence of platelets to leukocytes and endothelial cells but also enhances the expression of TF on monocytes,[14] and of platelet-bound TF further rules out a possible role of platelet activation in the postoperative period. This observation confirms and extends previous findings documenting similar behavior of these 2 differently located markers of platelet activation after ADP stimulus in vitro in healthy subjects.[13]

Particular attention should be given to TF, one of the main contributors of the early postsurgical hemostatic activation phase, a major determinant of atherosclerotic plaque thrombogenicity,[15] and the most likely mechanism of early saphenous vein graft failure caused by graft thrombosis.[16] Interestingly, cell-associated TF did not sensibly change in the perioperative period. Four days after the operation, however, TF circulating in plasma significantly increased, in accordance with previous findings from our group documenting a trend toward increased levels of this variable occurring a few days after CABG.[1,5] Circulating TF antigen is detectable in blood from healthy subjects, and increased levels of this protein are found in patients with cardiovascular disease, sepsis, and hematologic and coagulation disorders.[17] It recognizes several different cellular sources (eg, disrupted atherosclerotic plaques, platelets, apoptotic leukocytes, and endothelial cells), can circulate freely in the blood or in the form of cell-derived microparticles, and might contribute to thrombus propagation,[17-19] even though, depending on clinical situations, it might or might not bear procoagulant properties.[19]

The increase in soluble TF levels, which is common to CABG and OPCAB, might at least in part explain the increase in thrombin generation markers previously described from our group that occurs in the postoperative period of both CABG[1,2,5] and OPCAB[5] operations and that lasts up to 30 days after the operation. A possible explanation for the less protracted increase in TF with respect to thrombin generation markers is that TF activity of blood might need to be rapidly modulated and confined outside of the flowing blood or inside of the cells in contact with it to avoid excessive coagulation activation. In fact, it has been previously described that sensible amounts of TF might exist in an encrypted nonfunctional form on microparticles,[20] which are a heterogeneous family of small membrane vesicles released from different cells (eg, endothelial cells, mono-

cytes, granulocytes, and platelets) on activation or during apoptosis and act as transcellular effectors showing, on certain conditions, highly procoagulant features.[21,22]

Finally, no differences were detected between on-pump and off-pump operations in all the markers studied, with the exception of intraoperative higher levels of soluble P-selectin in patients in the CABG group, with the postoperative behavior of this marker being very similar in both groups. This adds further support to the hypothesis that, after an early phase of coagulation activation, where differences in some hemostatic variables do occur, CABG and OPCAB show a very similar pattern of biologic pathways activation not related to CPB and probably caused by the surgical trauma common to both procedures.[5]

In conclusion, cell-associated TF and P-selectin do not contribute to the postoperative procoagulant response that occurs in the first month after coronary bypass surgery performed both on pump and off pump, whereas the postoperative increase of plasma TF levels is the common mechanism that might in part explain the postoperative prothrombotic state occurring in low-risk patients undergoing uneventful CABG and OPCAB. It is possible, however, that different scenarios might occur in patients with important thrombotic or bleeding complications.

There is the need for future studies addressing the behavior of hemostatic variables in patients with thrombotic or hemorrhagic postoperative complications.

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Electronic Appendix

Materials

Adenosine diphosphate (ADP) and *Escherichia coli* lipopolysaccharide were from Sigma. Fluorescein isothiocyanate (FITC)–labeled monoclonal antibody (mAb) anti-human TF was from American Diagnostica. FITC-conjugated anti-human P-selectin mAb and phycoerythrin (PE)-conjugated anti-human glycoprotein IIb/IIIa mAb (CD41) were from Instrumentation Laboratories. PE-conjugated anti-human CD14 and FITC- and PE-labeled IgG1 isoantibodies were from Becton Dickinson.

Platelet Analysis by Means of Flow Cytometry

Platelet-associated TF and P-selectin expression were determined in whole blood (WB) by means of flow cytometry. Levels of the proteins were assessed both under resting conditions and on stimulation with ADP. Briefly, immediately after collection, 5 μL of blood was stimulated for 15 minutes at room temperature with ADP (10 μmol/L, stimulated sample) or vehicle (control sample) without stirring. Then samples were incubated for 15 minutes with saturating concentrations of the specific FITC- or PE-conjugated antibodies and analyzed with a flow cytometer (FACSCalibur, Becton Dickinson) equipped with a 15-mW, air-cooled, 488-nm argon-ion laser. Leukocytes were excluded with a combination of size- and platelet-specific marker (CD41) gating. The FITC-positive events (TF and P-selectin) were determined in 10,000 $CD41^+$ platelets per sample. Isotype control mAbs were used in each experiment to determine nonspecific background mAb binding. Mean FITC fluorescence intensity was calculated from fluorescence histograms for the gated population, and data were analyzed with a computer (CELLQuest software, Becton Dickinson). The results are expressed as the percentage change from baseline (Figures 1 and 2) and as mean fluorescence intensity (MFI; Table E2).

Monocyte Analysis by Means of Flow Cytometry

For flow cytometric determination of monocyte TF expression, 500 μL of unstimulated WB and 500 μL of WB stimulated with lipopolysaccharide (10 ng/mL) were incubated for 5 hours at 37°C without stirring. Then samples were fixed with 1% formaldehyde for 30 minutes at 4°C and washed twice. The fixed samples were incubated for 30 minutes with saturating concentrations of PE-labeled anti-CD14 and FITC-labeled anti-TF and then resuspended in 700 μL of lysis buffer and analyzed by means of flow cytometry. The TF-positive events were determined in 3000 $CD14^+$ monocytes per sample. Isotype control mAbs were used in each experiment to determine nonspecific background mAb binding. Mean FITC fluorescence intensity was calculated from florescence histograms for the gated population, and data were analyzed with a computer (CELLQuest software, Becton Dickinson). The results are expressed as percentage change from baseline (Figures 1 and 2) and as MFI (Table E2).

Plasma TF and P-selectin

Plasma TF and P-selectin levels were determined by means of enzyme-linked immunosorbent assay (ELISA; American Diagnostica and R&D Systems, respectively), according to the manufacturer's instructions.

TABLE E1. Clinical variables in the study population

Variable	CABG (n = 15)	OPCAB (n = 15)	*P* value
Age (y)	64 ± 1.4	65 ± 1.2	.58
Male sex (%)	10 (67%)	9 (60%)	>.99
Previous MI (%)	9 (60%)	6 (40%)	.47
Type I diabetes (%)	1 (7%)	2 (13%)	>.99
Type II diabetes (%)	3 (20%)	3 (20%)	>.99
COPD (%)	2 (13%)	4 (27%)	.65
Hypertension (%)	13 (87%)	10 (67%)	.39
Echocardiographic EF (%)	56 ± 1.5	58 ± 1.8	.39
Preoperative hematocrit (%)	39 ± 0.9	38 ± 1.2	.53
Diseased coronary vessels	2.9 ± 0.16	2.8 ± 0.13	.63
Distal anastomoses	3.2 ± 9.29	2.8 ± 0.23	.27
CPB time (min)	108 ± 5.5	–	–
Crossclamp time (min)	83 ± 3.7	–	–
2-h bleeding (mL)	97 ± 8.3	86 ± 6.4	.29
24-h bleeding (mL)	450 ± 21	467 ± 23	.58
Total bleeding (mL)	601 ± 15	639 ± 18	.11
Ventilation time (h)	6.4 ± 1.34	5.4 ± 1.61	.08

CABG, On-pump coronary artery bypass grafting; *OPCAB*, off-pump coronary artery bypass grafting; *MI*, myocardial infarction; *COPD*, chronic obstructive pulmonary disease; *EF*, ejection fraction; *CPB*, cardiopulmonary bypass.

TABLE E2. Tissue factor and P-selectin levels before, during, and after the operation in the CABG and OPCAB groups

		Baseline	Protamine	4 d	8 d	30 d
Plasma tissue factor (pg/mL)	CABG	157 ± 5.5	233 ± 7.3	275 ± 12.9	217 ± 5.5	157 ± 5.6
	OPCAB	145 ± 6.2	169 ± 3.9	263 ± 16.5	145 ± 5.5	150 ± 4.8
Unstimulated monocyte tissue factor expression (MFI)	CABG	0.95 ± 0.043	1.00 ± 0.040	0.93 ± 0.032	1.01 ± 0.043	0.93 ± 0.048
	OPCAB	0.95 ± 0.037	0.89 ± 0.029	0.93 ± 0.039	0.99 ± 0.043	0.87 ± 0.021
Stimulated monocyte tissue factor expression (MFI)	CABG	1.59 ± 0.094	1.76 ± 0.136	1.41 ± 0.080	1.54 ± 0.115	1.51 ± 0.061
	OPCAB	1.46 ± 0.115	1.25 ± 0.067	1.43 ± 0.107	1.57 ± 0.152	1.38 ± 0.048
Platelet tissue factor expression (MFI)	CABG	2.15 ± 0.208	1.76 ± 0.126	2.40 ± 0.246	2.20 ± 0.192	1.92 ± 0.147
	OPCAB	1.83 ± 0.150	1.67 ± 0.110	1.75 ± 0.152	1.78 ± 0.139	1.62 ± 0.166
ADP-induced platelet tissue factor expression (MFI)	CABG	3.37 ± 0.182	2.85 ± 0.139	3.53 ± 0.251	3.22 ± 0.190	2.68 ± 0.083
	OPCAB	3.15 ± 0.230	2.82 ± 0.203	2.63 ± 0.190	2.68 ± 0.142	2.61 ± 0.166
Platelet P-selectin expression (MFI)	CABG	0.94 ± 0.019	0.90 ± 0.027	0.97 ± 0.024	0.89 ± 0.024	1.03 ± 0.067
	OPCAB	0.90 ± 0.035	0.89 ± 0.037	0.86 ± 0.013	0.90 ± 0.021	0.98 ± 0.032
ADP-induced platelet P-selectin expression (MFI)	CABG	3.97 ± 0.496	3.04 ± 0.283	4.32 ± 0.267	4.01 ± 0.323	3.84 ± 0.393
	OPCAB	2.67 ± 0.416	2.32 ± 0.179	2.44 ± 0.182	2.60 ± 0.198	3.16 ± 0.390
Soluble P-selectin (ng/mL)	CABG	101 ± 3.7	131 ± 3.4	111 ± 3.5	133 ± 3.1	107 ± 2.5
	OPCAB	93 ± 3.0	64 ± 1.3	97 ± 2.4	113 ± 4.4	93 ± 2.1

CABG, On-pump coronary artery bypass grafting; *OPCAB*, off-pump coronary artery bypass grafting; *MFI*, mean fluorescence intensity; *ADP*, adenosine diphosphate.