Effect of Cardiopulmonary Bypass on Thrombin Generation and Protein C Pathway

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Objective: **The purpose of this study was to evaluate the mechanisms of cardiopulmonary bypass (CPB)-induced dysregulation between thrombin and its regulatory anticoagulant activated protein C (APC).**

Design: **A prospective observational cohort study.**

Setting: **A tertiary care university hospital and associated research laboratory.**

Patients: **Twenty patients undergoing elective coronary artery bypass surgery with (n = 10) or without CPB (n = 10).**

Interventions: **Blood samples were collected at 7 time points: preinduction; after heparin; 1 hour after the institution of CPB (or the completion of distal anastomoses in off-CPB group); after protamine; and at 0, 4, and 18 hours in the Intensive care unit (ICU). Samples were analyzed for prothrombin fragments (F1+2), thrombin-antithrombin complexes, protein C (PC), APC, soluble thrombomodulin (sTM), and soluble endothelial protein C receptor (sEPCR).**

Measurements and Main Results: **F1+2 levels increased significantly 1 hour after the initiation of CPB in comparison with baseline (2.7 ± 0.5 *v* 0.5 ± 0.2 nmol/L, $p < 0.001$) (mean ± standard deviation) and remained elevated until 4 hours after ICU admission ($p < 0.001$). In contrast, APC levels did not show any significant changes over time in either group. sEPCR, sTM, and PC levels did not change during CPB although sEPCR decreased significantly after the termination of CPB compared with baseline in the CPB group.**

Conclusions: **Exposure to CPB is associated with a distinct thrombin surge that continues postoperatively for 4 hours. The impaired ability to generate APC reflects a complex process that is not associated with increased levels of sEPCR and thrombomodulin during CPB. Further studies are required to evaluate the regulation of the host APC response in cardiac surgery.**

KEY WORDS: cardiopulmonary bypass, cardiac surgery, off–cardiopulmonary bypass surgery, thrombin, activated protein C, endothelial protein C receptor, thrombomodulin

EXPOSURE TO CARDIOPULMONARY BYPASS (CPB) is associated with a rapid and robust increase in thrombin generation.[1-3] Thrombin is a procoagulant and proinflammatory molecule that can trigger fibrin formation and platelet and endothelial cell activation.[4] Excessive generation of thrombin is associated with myocardial damage and hemodynamic changes.[3,5,6] Modulation of thrombin generation via patient-specific anticoagulation protocols has been associated with the preservation of coagulation factors and decreased transfusions after cardiac surgery.[7-9] However, the precise endogenous host response to the thrombin surge in cardiac surgery remains unclear.

Under physiologic conditions, thrombin binds to an endothelial surface membrane protein, thrombomodulin (TM),[10,11] and this catalyzes proteolysis of protein C (PC) to activated protein C (APC). The rate of APC activation is further enhanced when the substrate PC is bound to its receptor endothelial protein C receptor (EPCR).[12] It is the balance between the procoagulant activity of thrombin and its key regulatory anticoagulant APC that promotes a milieu of normal homeostasis.[13]

Anticoagulation with heparin can lead to rapid inactivation of APC.[14-16] Furthermore, the literature suggests that there is a marked delay in APC generation in response to thrombin production during CPB; this is associated with unfavorable hemodynamics, such as lower cardiac indices and higher systemic vascular resistances after CPB.[1] Impaired PC activation also is associated with increased myocardial neutrophil accumulation, suggesting its role in the regulation of inflammatory responses during reperfusion of human ischemic coronary circulation.[17] Thus, we hypothesized that the delayed APC formation in cardiac surgery leads to unimpeded effects of the thrombin surge associated with CPB, and we sought to evaluate the mechanisms of dysfunctional PC activation associated with exposure to CPB.

METHODS

The study was approved by the Institutional Health Sciences Research Ethics Committee. Twenty consecutive adult patients (10 in each group) scheduled for elective coronary artery bypass graft surgery with or without exposure to CPB consented and enrolled in this pilot study. Patients were excluded from the study if they were <18 years of age or were unable to give written consent. Other exclusion criteria included a history of any known coagulopathies; liver dysfunction; prior cardiac surgery; preoperative abnormal coagulation profiles; and recent exposure to heparin (unfractionated or low molecular weight), warfarin, clopidogrel, or other direct thrombin inhibitors in the preceding 14 days.

Anesthesia was induced with a combination of midazolam (0.02-0.05 mg/kg), fentanyl (4-6 μg/kg), and propofol (0.5-1.5 mg/kg) and maintained with isoflurane. Muscle relaxation was provided with rocuronium (0.5-1.0 mg/kg). Incremental doses of midazolam, fentanyl, and rocuronium were administered as per the discretion of the attending anesthesiologist. A baseline activated coagulation time (ACT) (Max ACT, Actalyke MAX-ACT; Array Medical, Somerville,

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NJ) was recorded after the induction of anesthesia and before surgical incision. Anticoagulation for surgery was initiated by unfractionated heparin (Hepalean; Organon, Toronto, Ontario, Canada) in the range of 300 to 400 U/kg body weight to achieve a target ACT >480 seconds in the CPB and the off-CPB group as per the standard of care at the authors' institution. Further heparin (5,000-10,000 U) was administered intraoperatively as necessary to maintain the target ACT. Tranexamic acid (bolus: 30 mg/kg, infusion 15 mg/kg/h) was administered intraoperatively to all patients as per the standard protocol. In the CPB group, patients underwent normothermic CPB using a membrane oxygenator and biocompatible circuits (Xcoating; Terumo, Ann Arbor, MI). The extracorporeal circuit was primed with 1,000 mL of Ringer's lactate, 400 mL of 10% pentastarch, 40 g of mannitol, 50 mL of 8.4% sodium bicarbonate, and 5,000 units of unfractionated heparin. After the termination of CPB or the completion of anastomoses in the off-CPB group, heparin was reversed with protamine sulfate (Sandoz; Canada Inc, Boucherville, Quebec, Canada) to return the ACT to within 10% of the baseline value. A further protamine bolus (50 mg) was administered at the discretion of the attending anesthesiologist and surgeon if little or no clot was seen forming on the surgical field.

Blood samples (9 mL) were drawn from arterial catheters at 7 time points: T1, before the induction of anesthesia (baseline); T2, 10 minutes after the heparin bolus; T3, 1 hour after the institution of CPB (or completion of distal anastomoses in the off-CPB group); T4, 10 minutes after protamine administration; T5, admission to the intensive care unit (ICU); T6, 4 hours after admission to the ICU; and T7, 18 hours after admission to the ICU. For each patient, 4.5 mL of the collected blood were immediately transferred into 15-mL polypropylene tubes containing 0.5 mL of 0.105 mol/L buffered trisodium citrate, and the remaining 4.5 mL was transferred into 15-mL polypropylene tubes containing 0.5 mL of 0.105 mol/L buffered trisodium citrate and 100 μL of 1 mol/L benzamidine HCl (20 mmol/L benzamidine final). The blood was spun at 1,500*g* for 10 minutes at 20°C, and the plasma was stored as aliquots in −80°C until further analysis. The citrated plasma samples are used for the PC antigen, prothrombin fragments (F1+2), thrombin-antithrombin complexes (TATs), the soluble endothelial protein C receptor (sEPCR), soluble thrombomodulin (sTM), and antithrombin-III (ATIII) activity, whereas the citrate/benzamidine plasma samples were used for APC assays. Benzamidine HCl, a reversible inhibitor of trypsin-like proteases, including APC, was necessary (at the time of blood collection) to block the irreversible inhibition of APC by plasma protease inhibitors and was removed during the APC assay wash steps, thus restoring the enzymatic activity of APC toward chromogenic substrates.

The APC assay was performed as described previously.[18] The total PC antigen was quantified in citrated plasma samples by a sandwich-type enzyme immunoassay (Affinity Biologicals Inc, Ancaster, Ontario, Canada). The levels of F1+2 and TAT in citrated plasma samples were quantified by the Enzygnost F1+2 micro kit and the Enzygnost TAT micro kit, respectively (Dade Behring Inc, Marburg, Germany). The levels of sTM in citrated plasma samples were quantified as described previously[19] except that the coating antibody was CTM 1009 and the detecting antibody was goat antimouse number 261 polyclonal antibody. The levels of sEPCR in citrated plasma samples were quantified as described previously[20] except that the coating and detecting antibodies were JRK 1535 and JRK 1495, respectively.

A total of 20 patients were included in the study (10 patients in the CPB group and 10 patients in the off-CPB group). Because this was a pilot study, no formal sample size calculation was performed. Data are presented as mean ± standard deviation. All hemostatic variables taken on CPB (T3) were adjusted for dilution using hematocrit (HcT) as the correction factor: $[HcT]_{initial} - [HcT]_{time\ point}/[HcT]_{initial}$. APC values of 0 (n = 3) were set to 0.25 for purposes of calculating F1+2/APC ratios. Between-group comparisons of demographic characteristics were performed using unpaired *t* tests when characteristics were normally distributed and the Wilcoxon rank sum test for data that were not normally distributed (eg, age, weight, protamine dose, ICU length of stay, and hospital length of stay).

Table 1. The Demographic Profile of Patients Enrolled in the Study

Variable	CPB	Off-CPB	*p* Value
Number of patients	10 (8 males)	10 (7 men)	
Age (y)	59.3 ± 6.9	70.2 ± 3.5	0.27
Weight (kg)	82.9 ± 5.2	78.7 ± 5.8	0.74
EuroSCORE	2.9 ± 0.6	5.1 ± 1.1	0.07
CPB time (min)	95 ± 18.3	—	
ACC time (min)	64.3 ± 14	—	
Initial heparin (U/kg)	326 ± 15	285 ± 20	0.12
Protamine (mg)	216.7 ± 22.7	201 ± 20.9	0.77
ICU LOS (h)	46.3 ± 13.2	27 ± 3.6	0.32
Hospital LOS (d)	7.3 ± 1.3	7.9 ± 1.6	0.73

Abbreviations: ACC, aortic cross-clamp; LOS, length of stay.

All biomarkers, with the exception of APC for which a square root transformation was used, were log transformed to improve normality. Two-way repeated measures analysis of variance with repeated measurements over time were used to evaluate differences between the 2 treatment groups (CPB and off-CPB) over time. In those cases in which the sphericity assumption was violated, the Huynh-Feldt–adjusted results were reported. When there was a statistically significant interaction (ie, F1+2, PC, TAT, F1+2/APC, sEPCR, and ATIII), a 1-way repeated measures analysis was performed within each group (CPB and off-CPB) to determine if significant changes occurred in 1 or both groups over time. When significant changes over time were found, the Dunnett *t* test was used to determine where there were significant changes from the preinduction level; *p* values <0.05 were considered statistically significant. All statistical analyses were conducted using SAS version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

The demographic profile and between-group comparisons of patients are shown in Table 1.

F1+2 levels (a sensitive marker of thrombin generation[21]) increased from preinduction levels (0.53 ± 0.2 nmol/L) in the CPB group and remained significantly elevated at 1 hour after the initiation of CPB (2.8 ± 0.5 nmol/L, $p < 0.001$), after protamine administration (2.7 ± 2.2 nmol/L, $p < 0.001$), upon admission to the ICU (3 ± 2.8 nmol/L, $p < 0.001$), and 4 hours thereafter (1.6 ± 0.2 nmol/L, $p < 0.001$) compared with baseline (Fig 1A). TAT complexes (a marker of thrombin inactivation) also increased significantly after the administration of heparin and remained elevated until 18 hours after ICU admission (Fig 1B). ATIII activity was elevated on CPB (147.9 ± 39.9, $p < 0.001$) and then decreased upon admission to the ICU (86.2 ± 19.2, $p = 0.017$) compared with baseline (107% ± 20.4% control). No significant changes were observed in ATIII levels in off-CPB patients compared with baseline (Fig 1C).

F1+2/APC ratios increased significantly from baseline (0.5 ± 0.3) at 1 hour after the initiation of CPB (3.8 ± 1.4, $p < 0.001$); after protamine administration (2.5 ± 3.7, $p = 0.036$); and at 0 hours (3.6 ± 4.5, $p = 0.001$), 4 hours (2.6 ± 1.4, $p < 0.001$), and 18 hours (2.0 ± 1.0, $p = 0.004$) after ICU admission in the CPB group (Fig 2). No significant changes were observed in the F1+2/APC ratios in the off-CPB group.