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Sustained heparin effect contributes to reduced plasma thrombin generation capacity early after cardiac surgery

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ABSTRACT

Introduction: Thrombin is a key component in the coagulation cascade, and impaired thrombin generation has been linked to increased bleeding after surgical procedures. The aim was to evaluate postoperative thrombin generation capacity in plasma after cardiac surgery, and its potential associations to activity of individual coagulation factors and heparin.

Material and Methods: Forty-eight coronary artery bypass grafting patients were included in a prospective observational cohort study. Thrombin generation capacity was analysed in plasma with calibrated automated thrombogram with tissue factor as activator before (baseline), and 2 h and 24 h after surgery. In addition, plasma activity of coagulation factors II, V, VII, VIII, IX, X, XI, XIII, were determined. Heparin effect was assessed by anti-Xa activity, APTT and thrombin time.

Results: Thrombin generation was markedly reduced 2 h after surgery compared to baseline. Peak levels decreased with median 74% (interquartile range 52–90), $p<0.001$, and endogenous thrombin generation potential decreased with 65% (43–86), $p<0.001$. Postoperative changes in endogenous thrombin generation potential correlated inversely to changes in anti-Xa activity ($r=-0.51$, $p=0.010$) and to changes in thrombin time ($r=-0.51$, $p=0.009$), but there were no correlations to changes in individual coagulation factor activity.

Conclusions: A marked reduction in thrombin generation potential was observed in the early postoperative phase after cardiac surgery. The decrease was independent of reductions in individual coagulation factor activity but correlated to heparin effects. The results indicate that a sustained heparin effect contributes to the postoperative reduction in thrombin generation capacity.

Introduction

Major surgery is one of the largest challenges for the hemostatic system, comprising surgical trauma, consumption of coagulation factors, hemodilution, and enhanced fibrinolysis. Cardiac surgery with cardiopulmonary bypass (CPB) imposes yet another challenge in form of blood exposure to non-endothelialized artificial surfaces and systemic heparinization. Subsequently, bleeding complications are not uncommon after cardiac surgery.

During cardiac surgery with CPB there is a massive increase in thrombin generation [1]. Thrombin is a pluripotent serine protease that plays an important role in hemostasis and thrombosis as well as in inflammation, vascular remodeling and angiogenesis [2]. Impaired postoperative thrombin generation capacity has been associated with increased blood loss after different surgical procedures [3–6].

Thrombin has a half-life of less than one minute, and is rapidly cleared from the circulation by antithrombin [2]. The short half-life makes it difficult to quantify thrombin concentration in vivo. Conventional coagulation analyses based on clotting times are not suitable because a clot occurs as soon as 5% of the whole amount of thrombin is produced, and the rest of thrombin remains thus undetected [7]. Therefore, indirect measurements of thrombin generation/inhibition by analysing prothrombin fragment 1 and 2 (F1.2), or thrombin-antithrombin complex (TAT), have predominantly been used to quantify thrombin generation.

Abbreviations: ACT, Activated clotting time; APTT, Activated partial thromboplastin time; BMI, Body mass index; CABG, Coronary artery bypass grafting; CAT, Calibrated automated thrombogram; CPB, Cardiopulmonary bypass; ECAT, External quality control of diagnostic assays and tests; ETP, Endogenous thrombin generation potential; FFP, Fresh frozen plasma; F1.2, Prothrombin fragment 1 and 2; PRBC, Packed red blood cells; TAT, Thrombin-antithrombin complex; TT, Thrombin time.

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In recent years, the calibrated automated thrombogram (CAT) method has gained interest. It assesses the plasma's whole enzymatic thrombin generating potential using tissue factor as initiator [8]. Reduced thrombin generation capacity has been reported after cardiac surgery [3,4,6,9,10] although there are publications reporting unchanged [11] or even increased thrombin generation capacity [12]. It has also been suggested that hemodilution and plasma activity of individual coagulation factors contributes to a reduction in postoperative thrombin generation [4–6,9].

We hypothesized that thrombin generation capacity as measured by CAT is reduced early after cardiac surgery and that the reduction is proportional to reductions in activity of individual coagulation factors. For this purpose, a prospective observational study was performed in CABG patients.

Material And Methods

Patients

Originally 59 consecutive patients undergoing first time CABG at Sahlgrenska University Hospital between September 2007 and February 2008 were included in a prospective observational non-interventional study. Two patients were excluded from analysis: one due to change of surgical approach (off-pump instead of on-pump CABG), and one because of on-going medication with clopidogrel at the time of surgery, not noticed at inclusion. Nine patients were excluded from the present analysis due to missing values in any of the thrombin generation measurements. The present analysis is thus based on 48 patients (mean age 64 ± 7 years, 77% males). Predefined exclusion criteria were acute operation, known bleeding disorder and on-going treatment with clopidogrel. The local medical research ethics committee approved the study protocol. Written informed consent was obtained from all study patients. Patient characteristics are given in Table 1.

Clinical management

Anesthesia was induced with 200–300 μg of fentanyl and 3–5 mg/kg of thiopentone, followed by 0.1 mg/kg pancuronium and maintained with sevoflurane. During CPB, anesthesia was maintained with propofol. The patients received 350 units unfractionated heparin/kg body weight. In addition, 10 000 units of heparin was added to the CPB circuit. The target activated clotting time (ACT) level was >480 seconds. After CPB, the heparin was reversed by administration of protamine sulphate (1 mg protamine/100 units of the initial heparin dose) [13].

The CPB circuit included a membrane oxygenator and roller pumps. Standard non-pulsatile CPB technique with moderate hypothermia (bladder temperature 34-36 °C), and hemodilution was used. The CPB circuit was primed with 1400 ml of Ringer-Acetate (Fresenius Kabi AB, Uppsala, Sweden), and 200 ml of Mannitol (150 mg/ml)(Fresenius Kabi AB). Cardioprotection was achieved with intermittent antegrade cold blood cardioplegia. Weaning off CPB was performed after rewarming to a bladder temperature of 36 °C.

Aspirin was not discontinued before surgery. None of the patients were treated with low molecular weight heparin or warfarin. Clopidogrel was discontinued at least three days before surgery. All patients received 2 g tranexamic acid intravenously at anesthesia induction and at the end of surgery. Aprotinin was not used in any of the study patients.

Table 1
Patient characteristics. Mean and standard deviation or number (%).

n	48
Male gender	37 (77%)
Age (years)	64 ± 7
BMI (kg/m²)	27 ± 3
Weight (kg)	80 ± 12
Euroscore	2.5 ± 1.8
Ejection fraction	0.59 ± 0.09
Preop clopidogrel	0
Preop warfarin	0
Preop LMWH	0
Preoperative aspirin	46 (96%)
CPB time (min)	70 ± 22
Aortic clamp time (min)	43 ± 14
Number of grafts	3.2 ± 0.9

Key: BMI = Body mass index, LMWH = Low molecular weight heparin, CPB = Cardiopulmonary bypass.

Study design

The following pre- and perioperative variables were registered: age, gender, body mass index (BMI), Euroscore, systolic ejection fraction, preoperative medication, number of grafts, CPB time and aortic clamp time. Postoperative bleeding was assessed as total amount of chest tube drainage during the first 12 postoperative hours.

Blood samples were collected at three time points: the day before surgery, and 2 and 24 hours after surgery. The preoperative blood samples were collected from an antecubital peripheral vein, and the postoperative samples from a non-heparinized radial arterial line, after discarding the first 10 ml of blood. Specimens were collected in sodium citrate tubes (0,13 M, 9 parts blood, 1 part sodium citrate), and centrifuged at 2000 g for 20 min. The supernatant was filled in separate tubes and freezed in dry ice for further analysis. Potential correlations between CAT values and coagulation factor activity were performed on both absolute values and intraoperative changes. Intraoperative change in the plasma variables was defined as a difference between value at 2 h after surgery and baseline value. To further illustrate the importance of reduced thrombin generation, patients were arbitrarily divided into two groups, one group with >90% postoperative reduction in ETP levels compared to baseline, and one group with <90% reduction.

Analyses

General

All analyses, except CAT and activated clotting time (ACT), were analyzed at the accredited coagulation laboratory at SahlgrenskaUniversityHospital. The laboratory participates in the ECAT foundation external quality assessment programme (www.ecat.nl). Hemoglobin concentration, hematocrit and platelet count were analyzed with clinical standard methods. CAT was analyzed at AstraZeneca R&D in Mölndal, Sweden. ACT was determined bedside on a HemocronJr II ACT + analyzer (ITC, Edison, NY, USA) 15 minutes after protamine reversal.

Coagulation factor activity

Fibrinogen (reference range 2.0-4.5 g/L) was measured by the modified method of Clauss. Factor II (FII) (reference range 70-130%), FV (reference range 60-140%), FVII (reference range 50-160%), FVIII (reference range 50-200%), FIX (reference range 45-190%), FX (reference range 70-130%) and FXI (reference range 60-140%) were determined using one stage clotting assays with specific factor deficient plasma samples on the instrument STA-R (DiagnostiaStago, Asnieres, France). Activity of FXIII (reference range 70-140%) was measured by a photometric method on the instrument Cobas Mira (Roche, Basel, Switzerland).

Indirect measurements of thrombin generation

F1.2 and TAT were measured using an enzyme-linked immunosorbent assay technique with commercially available tests (Enzygnost® F1 + 2 micro and Enzygnost® TAT, Dade Behring, Marburg Germany). The reference ranges are 70 – 230 pmol/L for F1.2 and 1.0 – 4.1 μg/L for TAT.