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Measurement of thrombin generation intra-operatively and its association with bleeding tendency after cardiac surgery

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ABSTRACT

Introduction: Patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) are susceptible to haemostatic disturbances. Monitoring the haemostatic capacity by conventional clotting tests is challenging.
Materials and Methods: Thrombin generation (TG) by Calibrated Automated Thrombography, clotting tests and tissue factor pathway inhibitor (TFPI) measurements were performed to describe the relationship between haemostatic changes and alterations in these tests. Blood samples were collected before, during and after CPB. Furthermore, it was investigated whether TG measured intraoperatively, is associated with increased risk of bleeding postoperatively.
Results: TG diminished significantly ($p < 0.01$) after heparinization in the presence and absence of platelets (37% and 50%) compared to baseline. After the start of CPB, TG elevated and persisted till the end of surgery but remained lower than preoperatively. Activated clotting time increased after heparinization and after the start of bypass compared to baseline (400% and 500%). Anti-FXa activity reduced on the start of CPB compared to the level after heparinization, to almost the baseline value following protamine reversal of heparin. The plasma levels of total and free TFPI elevated 9 and 14 fold during bypass and remained after protamine administration higher than preoperatively. Plasma D-dimer levels reduced ($p < 0.01$) when bypass started. However, a marked elevation was observed in the following time points. TG in platelet-rich plasma measured after heparinization and after the start of CPB associated ($p < 0.05$) with postoperative blood loss.
Conclusions: TG can be determined during CPB despite the high heparinization level, it reflects the haemostatic capacity better than clotting-based assays and might better predict bleeding when performed intraoperatively.

Introduction

Patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) are susceptible to disturbances in the haemostatic system related to use of extracorporeal circulation. It is well known that despite of high doses of heparin sufficient thrombin is generated [1,2]. Haemodilution causes reduction in plasma concentration of coagulation factors and platelet counts to decrease to about 50% of preoperative levels [3]. Hypothermia is related to both plasma coagulation factors and platelet dysfunction [4]. Consumption is caused by tissue injury, contact activation with the artificial surfaces of the extracorporeal circuit and the transfusion of shed pericardial blood [5]. Activation of both intrinsic and extrinsic pathways results in excessive thrombin generation and fibrinolysis [6]. High doses of unfractionated heparin are administered to create sufficient anticoagulation during CPB, and are routinely neutralized by the administration of protamine sulfate. Heparins exert their anticoagulant activity by accelerating the inhibitory action of antithrombin (AT) on factor Xa and thrombin. Besides this, heparin augments the regulation of tissue factor dependent coagulation by releasing tissue factor pathway inhibitor (TFPI).

All of these aspects are causing defects in the coagulation system, and therefore, it is challenging to monitor the haemostatic capacity of a cardiac surgical patient in a proper way. The utility of the conventional coagulation tests for this purpose is poor because 1) these clotting tests are performed in platelet-poor citrated plasma, excluding the interaction between platelets with (pro- and anti-) coagulation factors; 2) the tests terminate with endpoints that occur when less than 5% of thrombin is formed [7], and 3) they show a weak relationship with heparin concentrations [8]. Moreover, these tests are poor predictors of perioperative bleeding [9], and a thrombotic tendency is not reflected in a shortened clotting time [10].

Abbreviations: CPB, cardiopulmonary bypass; TG, thrombin generation; CAT, calibrated automated thrombography; TFPI, tissue factor pathway inhibitor; AT, antithrombin; CABG, coronary artery bypass grafting; ACT, activated clotting time; UFH, unfractionated heparin; PPP, platelet poor plasma; PRP, platelet rich plasma; BSA, bovine serum albumin; ETP, endogenous thrombin potential; PT, prothrombin time; aPTT, activated partial thromboplastin time; AUC, area under the curve; ROC, receiver operating characteristic; aXa, anti-factor Xa; SD, standard deviation; PRC, packed red cells; FFP, fresh frozen plasma; PLT, platelets.

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Because of the lack of a standard for measuring haemostatic capacity and the limitations of the conventional haemostatic measurements there is a need for tests that reflect better the haemostatic capacity of the patient's plasma [11]. Measuring thrombin generation, by calibrated automated thrombography (CAT), could be such a test. It reflects much, if not all, of the overall function of the blood clotting system [12]. All studies that used TG thus far showed high TG in all known prothrombotic conditions and predisposes for (re-)thrombosis [13–15]. TG is low in haemophiliac patients and restored by substitution therapy [13,15]. Moreover, recent clinical studies have demonstrated that the CAT is able to identify patients who are at an increased risk of bleeding after cardiac surgery [9,16].

In this study we measured thrombin generation by CAT and conventional clotting tests in patients who underwent cardiac surgery with CPB, in an attempt to describe the relationship between the changes in haemostatic equilibrium during surgery and the changes in these tests. In addition, we investigated whether thrombin generation measured intraoperatively when patients are already heparinized, is associated with an increased risk of bleeding after surgery.

Materials and methods

Study population

The study was approved by the local ethics committee, the procedures followed were in accordance with the institutional and national ethical standards and with the Helsinki Declaration, and written informed consent was obtained from participating patients. In total, 30 male patients undergoing elective first-time coronary artery bypass grafting (CABG) were enrolled. Exclusion criteria were age < 18 years, use of preoperative anticoagulation and platelet antagonists (excepting aspirin) within the preceding 5 days, known coagulopathy, impaired renal function, liver diseases resulting in elevated liver function tests and redo surgery.

Clinical management

General anaesthesia was induced using weight related dosing of sufentanil and etomidate, and muscle relaxation was achieved with pancuronium bromide. General anaesthesia was maintained during surgery using propofol. An initial dose of 300 IU/kg of body weight of heparin (Heparin Leo, Leo Pharmaceutical Products BV, Weesp, the Netherlands) was injected into a central venous line before initiation of CPB. The kaolin activated clotting time (ACT) was measured and, if the value was ≥400 s, CPB was initiated. If necessary, additional heparin was added. At the end of CPB, heparin was reversed by protamine chloride (Valeant Pharmaceuticals, Eschborn, Germany) at a 1:1 ratio of the loading dose.

All components of the CPB system were poly-2-methoxyethylacrylate coated (Terumo). The priming of the CPB circuit included 1,300 ml of 4% gelofusin, 200 ml 20% mannitol, 100 ml 20% human albumin, 50 ml 8.4% $NaHCO_3$. and 6,500 IU unfractionated heparin (UFH). Retrograde autologous priming was used in most of the cases to reduce the priming volume by 200–500 ml, resulting in less haemodilution. Normothermic perfusion (36 °C) was used during CPB. Pericardial, pleural and residual blood of the CPB circuit after termination of CPB was drained and washed with a cell saver device. The transfusion trigger during CPB was set at a haematocrit below 23%.

Study design

Preoperative data collection included demographics and aspirin use preoperatively. The following variables were perioperatively registered: time on bypass, aortic clamp time, medication, infusion volumes, transfusion requirements, amount of packed red cells processed with the cell saver. Postoperatively, blood loss, determined by chest tube drainage after closing the chest, was measured until 20 hours after surgery.

Blood samples were collected at six time points: T1) pre-bypass before heparin administration, T2) pre-bypass after heparin administration, T3) during CPB, after placing the aortic clamp and cardioplegia administration, T4) 30 minutes after the start of CPB, T5) end of CPB, T6) 5 min after protamine administration. These time points are representative of changes in the haemostatic equilibrium and provide more information on the responses to such changes. Blood samples before (T1-T2) and after CPB (T6) were withdrawn from the arterial line, after discarding the first 10 ml. The other samples (T3-T5) were drawn from heart lung machine.

Thrombin generation by calibrated automated thrombography

Arterial blood samples were collected into trisodium citrate and analyzed with CAT as previously reported [17]. Polybrene (Janssen Chimica, Beerse, Belgium) was used to neutralize heparinized samples, in order to explore the underlying thrombin generating capacity after heparinization and during CPB (T2-T5). Polybrene was used at 0.03 mg/mL (final concentration) to fully neutralize the effect of UFH. The neutralizing effect of polybrene was investigated at varying heparin concentrations in normal pooled plasma (data not shown). TG was measured both in platelet-rich plasma (PRP) and in platelet-poor plasma (PPP). TG in PRP was performed within 1 h after blood withdrawal. PPP was stored at -80oC until further analysis. The CAT assay was measured in a prewarmed plate fluorometer (Ascent reader, Thermolabsystems OY, Helsinki, Finland). In the PPP measurements, 10 μl of tissue factor (Innovin, Dade-Behring, Marburg, Germany) and phospholipids (Avanti Polar Lipids Inc. Alabaster, AL, USA) in Hepes buffer containing 5 g/ml bovine serum albumin (BSA) were added to each well, together with 10 μl of buffer with or without polybrene. The final concentrations of tissue factor an phospholipids were 5 pM and 4 μM, respectively. For PRP, the final concentration of tissue factor was 1 pM. Thrombin generation was initiated by adding 20 μl of Z-Gly-Gly-Arg-aminomethylcoumarine (2.5 mM) (Bachem, Basel, Switzerland) and CaCl2 (100 mM) in Hepes buffer containing 60 mg/ml BSA). Data were analyzed using ThrombinoscopeTM software (Thrombinoscope bv, Maastricht, the Netherlands). CAT parameters which are used to determine correlation of TG with postoperative blood loss are: 1) lag time (min): the initiation phase of clotting and equals the clotting time; 2) peak height (nM): the maximal amount of thrombin formed; 3) endogenous thrombin potential (ETP) (nM*min): the area under the TG curve; and 4) time to peak (min): the time needed to achieve the peak height. The general form of the thrombogram in the different conditions is shown in Fig. 1.

Routine laboratory tests and TFPI measurement

Besides CAT measurements, ACT (kaolin-activated, Hemotec ACT II Automated Coagulation Timer, Medtronic, Inc. Minneapolis, MN, USA) and laboratory parameters haematocrit, haemoglobin, thrombocytes (Coulter LH750 hematology analyzer, Beckman Coulter Inc. Miami, USA), fibrinogen, antithrombin (Siemens Berichrom Antithrombin III kit, Marburg Germany), D-dimers, prothrombin time (PT) and activated partial thromboplastin time (aPTT) were determined to assess patient's hemostatic profile. Anti-factor Xa (aXa) was also measured in all samples, using a competition method without addition of exogenous antithrombin (Biophen Heparin (LRT), Hyphen Biomed, Neuville sur Oise, France).

Plasma level of free and total TFPI was determined by use of ELISA (Asserachrom® free TFPI and Asserachrom® total TFPI, Diagnostica Stago, Asnières, France). Assays were performed according to manufacturer's instructions.