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Full Length Article

Hemostatic alterations during coronary artery bypass grafting

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ABSTRACT

Introduction: The origination of blood loss after cardiac surgery is not fully explained, but is related to operation trauma and use of cardiopulmonary bypass (CPB). However, the extent of their contribution is incompletely known and might differ between distinct operation procedures.
Materials and methods: Three groups of CABG procedures were studied: 1) off-pump coronary artery bypass surgery (OPCAB, n = 11) without CPB, 2) CABG with use of CPB (CABG, n = 11) and 3) CABG with use of CPB combined with aortic valve replacement (AVR, n = 11). Activation of coagulation and fibrinolysis was measured at various time points by flow cytometry, platelet aggregometry, thrombelastography, the Nijmegen Hemostasis Assay, prothrombin fragment 1 + 2 and tissue plasminogen activator.
Results and conclusions: The use of CPB during cardiac surgery decreased platelet counts, clot strength, fibrinogen, hematocrit and albumin concentrations during the procedure. No perioperative platelet activation was observed and functional (collagen induced) platelet aggregation was transiently impaired, but recovered after surgery in all groups. Patients operated with use of CPB showed increased tissue plasminogen activator concentrations after reperfusion followed by minor and transient fibrinolysis. After all types of surgery coagulation parameters and platelet aggregation showed a rebound above preoperative levels. To conclude, no evident platelet activation, dysfunction or consumption was demonstrated. In patients using tranexamic acid the most prominent factor impairing hemostasis after CABG surgery was hemodilution associated with CPB.

1. Introduction

Bleeding necessitating blood transfusions or requiring exploration by reoperation is a common complication after cardiac surgery [1,2]. A bleeding of surgical origin is found in half of the patients undergoing reoperation [3]. In the remainder of patients there is diffuse bleeding. An unambiguous explanation for the origination of bleeding tendency has not yet been established. Ineffective hemostasis can be due to pre-existing coagulation factor deficiencies, drug-induced inhibition of hemostasis or surgery related acquired hemostatic defects [2,4]. Suggested causes of acquired hemostatic defects in cardiac surgery include usage of heparin, hemodilution due to priming fluids of the cardiopulmonary bypass (CPB) circuit [5], activation of clotting by the non-endothelial CPB surface, hypothermia, acidosis, tissue trauma, platelet dysfunction and excessive fibrinolysis [4,5–8]. The extent of the contribution of each of those factors remains unresolved.

To gain more insight in the hemostatic disorders that develop during cardiac surgery, while using tranexamic acid, we have measured activation of coagulation and fibrinolysis by the release of prothrombin fragment 1 + 2 and tissue plasminogen activator at several time points during and up to 5 days after surgery in patients undergoing elective coronary artery bypass grafting (CABG). Furthermore, flow cytometry, platelet aggregometry, thrombelastography (TEG) [5,6] and the Nijmegen Hemostasis Assay (NHA), for simultaneous measurement of coagulation, fibrinolysis and the interplay of both [7], were performed.

2. Material and methods

2.1. Patient population

This observational study included a total of 33 adult patients undergoing elective CABG surgery, equally divided into three groups; 1) off-pump coronary artery bypass surgery (OPCAB) without CPB, 2) CABG with use of CPB (CABG) and 3) CABG with use of CPB combined with aortic valve replacement (AVR). These three groups were chosen to compare the hemostatic effects of the surgery and tissue damage itself

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(OPCAB group), with the effects of the use of CPB (CABG group) and the additional contribution of longer CPB times (AVR group). Patients needing emergency surgery, with heart failure (left ventricular ejection fraction <35%) or a history of bleeding diathesis or coagulopathy were excluded.

The study was performed in accordance with the Declaration of Helsinki and relevant Dutch laws, and was approved by the hospitals' ethics committee. All patients provided written informed consent.

2.2. Clinical and surgical management

All CPB procedures were executed normothermic (CPB machine (Jostra Maquet, Marquet, Hirrlingen, Germany), with intermittent antegrade warm-blood cardioplegia and prevention of acidosis. Heparin was injected as single bolus (300 U/kg in both groups) and monitored using the activated clotting time (ACT, Hemochron Signature Elite; ITC; Pleasanton, CA, USA). Additional heparin (5000 U) was given if ACT fell below 400 s. Tranexamic acid was used during surgery in 32 patients, using a bolus of 15 mg/kg prior to surgery and a continuous infusion of 5 mg/kg during operation. Pump flow rates were settled between 2 and 2.5 L index with a systemic mean arterial pressure target of 50–70 mm Hg and systemic vasodilators or vasoconstrictors were used to maintain pressure between the ranges. After surgery heparin was neutralized with protamine sulphate (1000 IE heparin:10 mg protamine sulphate). All blood from the operative field was filtered, stored in a separate cell saver system (Electa; Sorin Group; Mirandola, Italy) and retransfused according to local standards at the end of the procedure. Blood transfusion practice was based on the transfusion guidelines of the American Society of Anesthesiologists and the Dutch Institute for Healthcare Improvement (CBO). It recommends RBC transfusion when the hemoglobin (Hb) level is <7 g/dl and advices against the use when the Hb is >9 g/dl. In case of the Hb is >7 g/dl and <9 g/dl, the transfusion trigger is determined by blood loss, cardiopulmonary reserve, age, comorbidity, and at the discretion of the anesthesiologist or intensivist. Patients were extubated when hemodynamically stable, rewarmed, awake, without surgical bleeding and with optimal blood gases. Antiplatelet medication and low-molecular-weight heparin were both (re)started on the first postoperative day. If indicated, vitamin K antagonists were started five days after operation.

Blood loss was determined by drainage from the pleural and mediastinal tubes immediately after surgery until 48 h postoperative. Chest tubes were removed when drainage was less than 20 ml per hour or 200 ml per 24 h.

2.3. Blood sample collection

Blood was sampled in citrate (0.105 M buffered trisodium citrate solution, BD Vacutainer, Plymouth, UK), EDTA (BD Vacutainer) and CTAD (containing theophylline, adenosine and dipyridamol, BD Vacutainer) tubes at various time points: preoperative (T0), before CPB or 30 min after start of operation (T1), 30 min into CPB or 30 min after heparinisation administration (T2), 5 min after reperfusion (removal of aortic cross-clamp) or 5 min after completion of the last anastomosis (T3), 1 h after protamine administration (T4), postoperative days 1 (T5) and 5 (T6). Baseline (T0) and T6 samples were obtained via venous puncture. At all other time points blood was drawn from the central venous line.

Standard blood tests hematocrit (Ht), complete blood count, albumin, fibrinogen (Clauss, Roche Diagnostics, Almere, The Netherlands), D-dimer (Roche Diagnostics), activated partial thromboplastin time (aPTT) and prothrombin time (PT) were performed at the hospital laboratory. Citrated whole blood was centrifuged at 2700 *g* for 10 min at 18 °C and the CTAD tubes were centrifuged at 4200 *g* for 15 min at 4 °C, aliquotted and stored at −80 °C until batch analysis. All samples were analyzed blinded for clinical data or patient outcomes.

2.4. Hemostasis parameters

2.4.1. Platelet tests

Immediately after collection, P-selectin expression, spontaneous and induced by adenosine diphosphate (ADP, 10–4 M) and Collagen Related Peptide (10 μg/ml), were determined by whole blood flow cytometry [6] (Beckman Coulter FC500 MPL, Beckman Coulter, USA), at all time points.

Light transmission aggregometry (Chrono-Log 490 aggregometer, Chrono-Log Corporation Havertown, PA) with ADP (4 μM), collagen (2.0 μg/ml) and arachidonic acid (1.6 mM) as activators was performed at T0, 2, 5 and 6 in platelet rich plasma.

2.4.2. Thrombelastography

Within 10 min after sampling whole blood thrombelastography (TEG^R 5000 Thrombelastograph® Hemostasis Analyzer System, Hemonetics Corporation, USA) analysis was performed at all time points using kaolin as activator. TEG parameters included the r value (intrinsic coagulation cascade activity), the α angle (speed of solid clot formation) and the maximal amplitude (clot strength, MA). The MA measurement was also separately performed for functional fibrinogen (FF MA), blocking GPIIb/IIIa receptors on platelets.

2.4.3. Coagulation and fibrinolysis

Prothrombin fragment 1 + 2 was measured at T0, 3, 5 and 6 by ELISA (Antibodies-Online, Aachen, Germany).

The Nijmegen Hemostasis Assay (NHA) was performed for simultaneous measurement of coagulation and fibrinolysis at T0, 3, 5 and 6 [7]. The NHA measures thrombin generation lag time (time between initiation and the start of thrombin generation, TGlt), time to peak thrombin generation (time at which the thrombin generation reached its maximal rate, TTP), thrombin peak height (maximal velocity of thrombin production, TPH) and area under the curve (thrombin generation capacity, AUC) for coagulation and the fibrin lysis time (FLT) and plasmin peak height (height of plasmin generation, PPH) for fibrinolysis.

Tissue plasminogen activator (t-PA) levels in CTAD plasma were determined at T0, 3, 5 and 6, using the Human tPA activity assay (Kordia, The Netherlands) which is not sensitive to tranexamic acid administration.

2.5. Statistical analysis

For descriptive purposes we used medians and ranges. Statistical significance was defined as $p < 0.05$. For comparison of several outcome variables between the 3 treatment groups at each time point and between time points, we used a linear mixed model with time and group and their interaction as categorical covariates and with compound symmetry as covariance model. All analyses were corrected for the following covariates: blood transfusions (i.e. red blood cells, fresh frozen plasma (FFP), platelet rich plasma and cell saver blood), preoperative acetylsalicylic acid and clopidogrel usage, dilution with colloids and crystalloids, and current value of hematocrit. The statistical analyses were performed using SPSS Statistics 20.0 software (IBM Corporation, Armonk, NY).

3. Results

3.1. Patient characteristics

Patient groups significantly differed on 2 baseline characteristics: logistic EuroSCORE and extracardiac arteriopathy, defined as any one or more of the following: claudication, carotid occlusion or >50% stenosis, previous or planned intervention on the abdominal aorta, limb arteries or carotids (Table 1). All other baseline variables, including laboratory, were comparable among groups.

As expected patients in the AVR group had the longest operation, CPB and aortic clamping time, the lowest minimum temperature and