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Fibrin formation is more impaired than thrombin generation and platelets immediately following cardiac surgery

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ABSTRACT

Introduction: Cardiac surgery performed on cardio-pulmonary bypass (CPB) may be complicated by coagulopathy and bleeding. This prospective observational study investigated the CPB-induced changes in thrombin generation, fibrin formation, and in the platelet component of the whole blood clot elasticity. The effects of haemostatic therapy with fresh frozen plasma (FFP) and platelet concentrate on these parameters were also evaluated.

Materials and Methods: In 90 cardiac surgery patients, thrombin generation was measured using the calibrated automated thrombogram, fibrin formation was assessed as the maximum clot elasticity of the fibrin-based clot in the thromboelastometry FIBTEM test (MCE_{FIBTEM}), and the platelet component was defined as the difference in maximum elasticity between the whole blood clot obtained through extrinsic activation and the fibrin-based clot (MCE_{EXTEM}-MCE_{FIBTEM}). Blood samples were collected before surgery, immediately after CPB, and after administration of FFP or FFP and platelet concentrate.

Results: Following CPB, the endogenous thrombin potential decreased to 93%, from median 1485 (interquartile range 1207, 1777) to 1382 (1190, 1533) nM*min ($P>0.05$), MCE_{FIBTEM} decreased to 62%, from 21 (19, 29) to 14 (12, 19) ($P<0.001$), and the platelet component to 73%, from 139 (119, 174) to 101 (87, 121) ($P<0.001$). Administration of 11 (10, 13) ml per kg of bodyweight (ml/kgbw) FFP (40 patients), or of 13 (10, 18) ml/kgbw FFP and 7 (5, 9) ml/kgbw platelet concentrate (18 patients) brought no statistically significant changes in these parameters.

Conclusions: Fibrin formation is more impaired than thrombin generation and the platelet component of the whole blood clot immediately after cardiopulmonary bypass.

Introduction

The haemostatic dysfunction seen in cardiac surgery performed on cardio-pulmonary bypass (CPB) has a multifactorial etiology: haemodilution, loss and consumption of coagulation factors and natural anticoagulants, and changes in platelet count and function [1,2]. Despite sparse medical evidence [3], management of perioperative bleeding in cardiac surgery is often based on transfusion of fresh frozen plasma (FFP) and platelet concentrates (PC) guided by laboratory assessment of prothrombin time (PT)/prothrombin time index (PTI) or activated partial thromboplastin time (aPTT) and platelet count, respectively. Noteworthy, the majority of non-bleeding patients undergoing cardiac surgery experience a decrease in platelet count and prolonged PT/increased PTI/increased international normalised ratio (INR), as well as prolonged aPTT [4]. However, these standard laboratory parameters provide inadequate guidance for timely and goal-directed haemostatic intervention. In addition, accumulating epidemiological data show that transfusion of allogeneic blood components may be associated with serious adverse events, such as increased frequency of infections, transfusion related acute lung injury, and increased mortality [5,6]. Hence, better understanding of the mechanisms of coagulopathy is likely to be clinically beneficial.

During recent years, use of global coagulation tests like calibrated automated thrombin generation and whole blood thromboelastometry has provided the opportunity to perform a more thorough investigation of overall haemostatic capacity in bleeding patients. In some countries, point-of-care whole blood thromboelastometry is used to direct haemostatic intervention [7,8]. This approach is derived from thromboelastography, pioneered by H. Hartert in the 1960 s; two devices (TEG® [Haemonetics Corp., Braintree, MA, USA] and ROTEM® [Tem International GmbH, Munich, Germany]) are currently available. Measurement of whole blood clot formation and quality in

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patients undergoing major cardiovascular surgery has demonstrated an important decrease in fibrin formation at the end of extracorporeal circulation [9,10]. Thrombin generation measurement has been explored in various scenarios, such as congenital bleeding disorders and anticoagulation [11,12], and there are isolated reports of decreased thrombin generation following CPB in patients undergoing cardiac surgery [13]. Currently, there is an ongoing important debate whether the predominant coagulation defect immediately after CPB is represented by abnormal thrombin generation [14] or decreased fibrinogen concentration [15] and impaired fibrin formation. In addition, it is well known that platelet count and function may be affected by CPB [16,17]. Improved haemostatic management of patients undergoing cardiac surgery requires more knowledge on the absolute and relative changes in thrombin generation, fibrin formation as well as the platelet component. Moreover, the effect of FFP or FFP combined with PC as haemostatic interventions for correcting fibrin formation, overall blood clot quality, and thrombin generation in bleeding patients following cardiac surgery are largely unknown.

The primary objective of the present prospective observational study was to investigate absolute and relative changes in thrombin generation, platelet component of whole blood clot elasticity, and fibrin formation in a clinically representative heterogenic group of patients undergoing cardiac surgery on CPB. The second aim was to investigate the effect of FFP or FFP combined with PC on thrombin generation, platelet component, and fibrin formation in patients experiencing perioperative bleeding. We tested the hypothesis that thrombin generation, platelet component, and fibrin formation reveal equal relative change during cardiac surgery. Furthermore, we challenged the hypothesis that FFP or FFP combined with PC does not change thrombin generation, platelet component or fibrin formation.

Materials and methods

Patient population and classification

Following Research Ethics Committee approval and informed consent, consecutive patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) from December 2005 to January 2006 were included in the study. Before the operation, the patients were thoroughly interviewed regarding intake of aspirin, non-steroidal anti-inflammatory drugs, glycoprotein (GP) IIb-IIIa antagonists, thienopyridines or antibiotics known to influence platelet aggregation. Exclusion criteria were non-elective or acute procedures, age less than 18 years, terminal illness, and intake of clopidogrel within 4 days before surgery.

Patients were sub-categorised according to the transfusion requirements for FFP and PC: i) no-transfusion group, ii) FFP group, and iii) FFP-PC group, with the assumption that the sub-groups would represent patients with minor and more pronounced haemostatic deficits.

Collection of blood samples

Blood samples were drawn from a radial artery catheter (20 Gauge) into Sarstedt collection vials (Sarstedt, Nuembrecht, Germany) containing trisodium citrate (3.13%) or EDTA as anticoagulant. Blood was sampled at baseline (at the beginning of the operation, before induction of the anaesthesia), at the end of CPB, and at the end of surgery. Haemostatic therapy with FFP or FFP combined with PC was administered in the time period after CPB and before the end of surgery. The citrated blood samples were centrifuged at 2000 g for 15 minutes to obtain platelet-poor plasma, which was stored at −70 °C until the analysis. EDTA blood was used for full blood count.

Thrombin generation

Thrombin generation was measured using a calibrated, automated thrombogram (Thrombinoscope BV, Maastricht, The Netherlands) [18]. After thawing in a water bath at 37 °C, the platelet-poor plasma samples were centrifuged for 10 minutes at 2000 g and for 5 minutes at 10000 g at room temperature. 20 µl of PPP-Reagent HIGH (Thrombinoscope BV, Maastricht, The Netherlands) containing tissue factor and phospholipid were transferred to each well of a 96-well microtiter plastic plate (Immulon 2HB clear 96-well, Thermo Electron), followed by addition of 80 µl platelet poor plasma. After a brief incubation, 20 µl of thrombin substrate (Fluo-Substrate, Thrombinoscope) were added automatically. All reagents were prewarmed to 37 °C. The final reaction mixture contained 20 pm tissue factor and 4 µm phospholipids. Continuous generation of thrombin was recorded on a Fluoroscan Ascent fluorometer (Thermo Electron Corporation, Vantaa, Finland). The measurements were performed in triplicate, with each well calibrated to a parallel well with a thrombin calibrator (Thrombin calibrator TS 20.0, Thrombinoscope). Following parameters were recorded: endogenous thrombin potential (ETP, calculated as the area under the thrombin generation curve), lag time, peak height and time to peak. ETP was selected as the primary endpoint for total thrombin generation.

Fibrin formation and platelet component

A four channel ROTEM device was used for the thromboelastometric analyses of the blood samples as previously described in literature [8]. The reagents and 300 µl blood were transferred into a single-use plastic cup which was set onto an oscillating plastic pin. The reduction in the pin's movement caused by the clot was mathematically transformed into clot firmness amplitude (mm) and plotted against time, resulting in the thromboelastometric trace. Clotting was induced by rabbit brain tissue factor (EXTEM, extrinsically activated test) and by rabbit brain tissue factor and cytochalasin D (FIBTEM, assessment of fibrin formation after platelet inhibition). 20 µl of 0.2 M $CaCl_2$ was added to each test for recalcification of the citrated blood sample. All reagents were obtained from TEM International (Munich, Germany). The following ROTEM parameters were recorded: clotting time (CT, time from the start of the test until an amplitude of 2 mm was detected), maximum clot firmness (MCF) and maximum clot elasticity (MCE). MCF and MCE are related as follows: MCE = (MCF*100)/(100-MCF). MCE_{FIBTEM} was selected as the primary outcome parameter for total fibrin formation, whereas the platelet component (MCE_{EXTEM} − MCE_{FIBTEM}) was selected as primary endpoint parameter reflecting the contribution of platelets to the elasticity of the whole blood clot [19].

Standard coagulation tests

Activated partial thromboplastin time, aPTT (APTT Kaolin, STAGO Diagnostica) and prothrombin time (PT)/prothrombin time index (PTI) (Neoplastin, STAGO Diagnostica) were determined on the STA-R analyzer (STAGO Diagnostica, Asnieres, France). Fibrinogen concentration was measured on the STA-R Evolution analyzer (STAGO Diagnostica & Roche, Germany) using the Clauss method and platelet count and haematocrit on the Sysmex XE-2100 (Roche Diagnostics, Mannheim, Germany).

Clinical management

Anaesthesia was induced with etomidate, fentanyl and cisatracurium. Patients received Ringer's solution and gelatine polysuccinate during induction. For maintenance of anaesthesia, sevoflurane was titrated to an end-tidal concentration of 1–2% until institution of CPB. For the duration of CPB, propofol in continuous infusion and additional boluses of fentanyl were administered. A bolus of heparin 400 IU/kg was