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Regular Article

The combination of recombinant factor VIIa and fibrinogen correct clotting *ex vivo* in patient samples obtained following cardiopulmonary bypass surgery

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ABSTRACT

Cardiac surgery involving cardio pulmonary bypass (CPB) may be associated with development of a coagulopathy that increases risk of bleeding. In the present *ex vivo* study we investigated the influence of fibrinogen and rFVIIa, alone or in combination, on whole blood coagulation thromboelastometry using pre- and postoperative blood samples from 18 consecutive adult patients undergoing CPB surgery. Dynamic thromboelastometric clotting profiles were recorded using citrated whole blood activated with trace amounts of tissue factor (Innovin®, final dilution 1:17000). Blood samples were collected before surgery (control) and postoperative samples were obtained following in vivo neutralization of heparin with protamine sulphate. All blood samples were treated with heparinase to ensure neutralization of possible residual heparin effect. The post-operative blood samples were spiked with buffer, rFVIIa (2 μg/mL), fibrinogen (1 mg/mL), or the combination of rFVIIa and fibrinogen. Despite neutralization of heparin, CPB surgery left a measurable coagulopathy that was thromboelastometrically characterized by prolonged onset of clotting, reduced maximum velocity of clot formation (MaxVel), and decreased maximum clot firmness (MCF). *Ex vivo* spiking of the postoperative samples with rFVIIa shortened the clotting time. Fibrinogen also shortened the clotting time and, in addition, improved the MaxVel, and MCF. Finally, adding the combination of rFVIIa and fibrinogen to the postoperative samples corrected all thromboelastometric parameters to the preoperative range. In conclusion, the correction of whole blood clotting abnormalities that occurs with rFVIIa and/or fibrinogen suggests that future clinical trials on treatment of bleeding during CPB surgery should study the haemostatic effect of fibrinogen or possibly the combination of rFVIIa and fibrinogen.

1. Introduction

During cardiopulmonary bypass (CPB) surgery a multifactorial coagulopathy may develop and this may contribute to peri- and post-operative bleeding. Possible causes include heparinization, surgical trauma, haemodilution, extracorporal circulation, consumption coagulopathy, increased fibrinolysis, and hypothermia [1–3]. Despite adequate neutralization of heparin with protamine sulphate at the end of surgery [4], haemostasis often remains compromised and this may contribute to severe bleeding, leading to surgical re-exploration in 3 to 4% [4,5]. Standard medical management of the coagulopathy includes transfusion of red blood cells, fresh frozen plasma, and platelets [6]. Clinical reports have also suggested a beneficial haemostatic effect of recombinant factor VIIa (rFVIIa) administration [7–9] or the administration of a fibrinogen concentrate or cryoprecipitate [10].

Bedside whole blood rotational thromboelastometry (ROTEM®) using activation with minute amounts of tissue factor is a sensitive method that may allow tailoring of haemostatic intervention in various coagulopathies and during surgery [11–13]. The ROTEM could possibly help predict the clinical response to various haemostatic agents [14–18]. Recently, Tanaka et al reported an improvement in ROTEM parameters after addition of rFVIIa (1.5 μg/mL) and fibrinogen (1 mg/mL) alone or in combination to postoperative blood samples obtained following CPB surgery in seven patients, although no preoperative control or other details revealing a coagulopathy were shown [19].

In the current *ex vivo* study, done on whole blood samples obtained from patients before and after CPB surgery, we tested the

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ex vivo effect of rFVIIa, fibrinogen, and the combination of both on the abnormal thromboelastometric coagulation pattern that developed during surgery.

2. Materials and Methods

2.1. Study subjects

We studied eighteen consecutive adult patients undergoing cardiopulmonary bypass surgery at the Landspitali University Hospital in Reykjavik, Iceland. None of the patients were treated with aprotinin or tranexamic acid during surgery. Patients with known congenital thrombotic or haemostatic disorders were not eligible. The median age of the study population was 68 years (range 39-82). Twelve patients underwent coronary artery bypass grafting (CABG), two aortic valve replacement (AVR), three CABG + AVR, and one mitral valve repair. Nine patients had stable angina pectoris (AP) and underwent elective surgery whereas the other 9 patients presented with unstable AP and had expedited surgery. All patients with unstable AP received low molecular weight heparin (enoxaparine 80 mg) q12 hours until the day of operation. According to standard hospital procedure, patients undergoing elective surgery were recommended to discontinue aspirin prior to surgery and the aspirin was discontinued between 2 - 18 days prior to surgery (median = 8 days).

2.2. Blood sampling

Two sets of blood samples were obtained from the distal lumen (16 Ga) of the non-heparinized central venous catheter (Arrow-Howes™ Quad-Lumen, Arrow International Inc. Reading, PA, USA). The first set was obtained immediately after the patients were anaesthetized but prior to initiation of surgery and the second set 15 minutes after termination of CPB and full reversal of heparin (Leo® Pharma A/S, Copenhagen, Denmark) with protamine sulphate (Leo® Pharma A/S, Copenhagen, Denmark). The first 10 ml of aspirated blood were discarded to minimize pre-activation of the blood. For coagulation and platelet analyses we used 5 ml 3.2% sodium citrate Becton-Dickinson vacutainer® tubes (Becton Dickinson, Belliver Industrial Estate, Plymouth, United Kingdom), whereas 4 ml BD EDTA vacutainer tubes were used for measurement of the complete blood count.

2.3. Reagents and buffers

As a buffer control we used HEPES 20 mM, NaCl 150 mM, pH = 7.4 (Bie & Berntsen A-S Herlev, Denmark). Recombinant factor VIIa (rFVIIa, NovoSeven®, Novo Nordisk, Bagsvaerd, Denmark) and fibrinogen concentrate (Haemocomplettan®, CSL Behring, Marburg, Austria) were obtained from the manufacturer. The HEPTEM® reagent from Pentapharm, Munich, Germany was used as source of heparinase. The tissue factor (TF) source was Innovin® from Dade Behring, Marburg, Germany.

2.4. Whole blood coagulation analyses

Dynamic whole blood clot formation profiles were recorded by a ROTEM® Thromboelastometry Coagulation Analyzer (Pentapharm, Münich, Germany). The analytical methodology adopted is described elsewhere [20]. In brief, citrated blood samples rested for 30 minutes at ambient temperature. The reaction mixture contained 280 µl of citrated whole blood + 10 µl of heparinase + 20 µl buffer or drug (final added concentrations: rFVIIa 2 µg/mL corresponding to a 90 ug/kg dose and/or fibrinogen 1 mg/mL corresponding to a 3 g dose in a 80 kg person) + 20 µl TF and $CaCl_2$ 200 mM. All results shown are based on means of duplicate experiments. The thromboelastometry measurement of whole blood clot formation was based on activation with minimal amounts of tissue factor (final reaction mixture dilution 1:17,000). Assessment of whole blood clot formation was based on standard thromboelastometry parameters such as the clotting time (CT) and the maximum clot firmness (MCF). In addition, the ROTEM® raw data were processed using the DyCoDerivAn™ software (AvordusoL, Risskov, Denmark) providing dynamic velocity profiles and derived parameters such as the maximum velocity (MaxVel) and time to maximum velocity (t, MaxVel) of clot formation [20]. The CT characterizes the initiation phase of whole blood clot formation. The MaxVel and t, MaxVel, define the propagation phase of whole blood clotting. The stabilization phase is expressed by the MCF and is predominantly sensitive to platelet count and level of fibrinogen [21].

2.5. Whole blood platelet function analyses

Whole blood platelet function was evaluated by detection of PFA-100 closure times using the Platelet Function Analyser PFA-100® (Dade-Behring, Marburg, Germany) as well as Dade® PFA collagen/epinephrine (CT c/epi) and Dade® PFA Collagen/ADP (CT c/ADP) test cartridges. In brief, 800 µL of citrated whole blood were pipetted into the test cartridge. By vacuum pressure, the whole blood flows through a micro-aperture in the cartridge membrane that is coated with collagen and either ADP or epinephrine. The instrument monitors reductions in the blood flow rate as the platelets form a haemostatic obstruction in the aperture. Arrest of blood flow is denoted as closure time and the maximal value obtainable is 300 s.

2.6. Other laboratory coagulation analyses

The STA-R coagulation analyzer (Diagnostica Stago, Asnieres, France) was used for the follwing plasma coagulation, chromogenic tests and immunoturbidometric tests. The activated partial thromboplastin time (APTT, sec), prothrombin time (PT, sec), fibrinogen concentration (g/L), thrombin time (TT, sec) were measured using Platelin® LS (BioMérieux USA), Tissue factor STA® Néoplastine® CI plus, STA®-FIBRINOGEN 5, and STA®-Thrombin, respectively. The activity of antihrombin, protein C, and antiplasmin was measured using STA®-STACHROM® AT III, STA®-STACHROM® PROTEIN C, and STA®-STACHROM® ANTIPLASMIN, respectively. D-dimer was determined using the STA®-LIATEST®D-DI reagent. The reagents were obtained from Diagnostica Stago unless stated otherwise.

2.7. Statistical considerations

The distribution of data was evaluated using histograms and Q-Q-plots. Data did not follow a Gaussian distribution, hence data comparison was performed using the non parametric Wilcoxon signed rank test for paired data. Results are shown as median (range). Statistical significance was defined by a p-value < 0.05.

2.8. Ethical considerations

The study was approved by the Ethical Committee of the Landspitali University Hospital in Reykjavik, Iceland and the Data Protection Authority of Iceland. Informed consent was obtained from all participants prior to surgery.

3. Results

3.1. Clinical data

The median time on cardiopulmonary bypass was 102 minutes (range 45-206). The median cross clamp time was 54 minutes (range 21-153). The median lowest core body temperature was 35 °C (33.2-35.7). The median blood loss during the operation measured based on suctioned waste and weight of used gauzes and sheets was 1055 mL (10 – 3750). In the 18 patients, there was a significant positive correlation between the