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The study of the thrombin generation mechanism and the effect of low molecular weight heparin as thromboprophylaxis in patients undergoing total knee and hip replacement

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ABSTRACT

Introduction: The recommended duration of post-operative Low-Molecular-Weight-Heparins (LMWHs) thromboprophylaxis in Total-Hip-Replacement (THR) and Total-Knee-Replacement (TKR) surgery is controversial. Our aim is to study the thrombin generation (TG) modifications induced by surgery and to evaluate the effect of LMWH on TG during and after the recommended duration.

Patients/Methods: Thirty-one patients received 4000 IU anti-Xa/day of enoxaparin, 8-hours post-operatively (15 THR for 30-days and 16 TKR for 15-days). TG assay sensitive to enoxaparin was performed, pre-operatively (D0), 7-hours post-surgery (D1), 8-days post-surgery (D8), and 2-days after thromboprophylaxis withdrawal (D32 and D17), evaluating: lag-time, endogenous thrombin potential (ETP), peak amount of generated thrombin (Peak), time-to-Peak (tt-Peak), and the Mean-Rate-Index [MRI = Peak/(tt-Peak–lag-time)].

Results: TKR surgery decreased lag-time and tt-Peak and increased MRI on D1 vs D0 ($p < 0.05$). In contrast, THR did not significantly modify TG. Enoxaparin effectively reduced thrombin generation in both groups. Thromboprophylaxis withdrawal resulted in rebound increase of TG in the TKR patients (ETP, Peak & MRI significantly increased on D17 vs D0; $p < 0.05$, and vs. D1; $p < 0.05$) but not in THR patients. Variability in the response to enoxaparin was observed among patients of the same group.

Conclusions: TKR surgery is more thrombogenic than THR surgery. In THR patients TG was efficiently inhibited by 30-day thromboprophylaxis, whereas, in TKR patients treated for 15-days TG was not effectively inhibited. Individual variability of the response to enoxaparin was observed in both groups revealing some form of biological resistance to enoxaparin. TG assay may represent the breakthrough step to efficient antithrombotic strategy in clinical settings with high thrombotic risk.

Introduction

Elective Total Hip Replacement (THR) and Total Knee Replacement (TKR) are common major orthopaedic interventions associated with a high risk of venous thromboembolism (VTE) which persists for several weeks after the surgery [1,2]. In the absence of thromboprophylaxis, the respective incidence of total and proximal deep vein thrombosis (symptomatic and asymptomatic) is 42–57% and 18–36% in THR patients, respectively, and 41–85% and 5–22% in TKR patients, respectively. Fatal Pulmonary Embolism (PE) occurs in 0.1–2% of THR patients and in 0.1–1.7% of TKR patients [1,2]. Routine administration of a fixed dose of low molecular weight heparins (LMWHs), fondaparinux or orally active direct factor Xa or thrombin inhibitors is strongly recommended in patients undergoing THR or TKR [1,2]. LMWHs present predictable pharmacokinetics allowing effective thromboprophylaxis with a single daily subcutaneous injection [3]. Although LMWHs represent a substantial improvement in the antithrombotic strategy, about 10% to 20% of patients undergoing major orthopaedic surgery (MOS) who receive the recommended prophylaxis with LMWH may still present asymptomatic VTE [4–6].

Abbreviations: THR, Total Hip Replacement; TKR, Total Knee Replacement; VTE, venous thromboembolism; LMWHs, low molecular weight heparins; tt-Peak, time necessary to Peak; ETP, endogenous thrombin potential; MRI, mean rate index.

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The LMWHs are pleiotropic anticoagulant drugs exerting their antithrombotic activity by inhibiting thrombin generation via the antithrombin dependent inhibition of serine proteases (factor Xa and to a lesser extent by inhibiting factor IIa, factor IXa [7–10]. In addition, LMWHs inhibit factor VIIa generation and activity and induce Tissue Factor Pathway Inhibitor (TFPI) release that contributes to their antithrombotic effect [11–13]. On the other hand, the longer glycosaminoglycan chains of LMWH bind non-specifically to plasma proteins, and part of its antithombotic activity is neutralized by platelet factor 4 (PF4) released from activated platelets [14]. Activation of platelets, leukocytes and endothelium cells is enhanced during the perioperative period, inducing hypercoagulable state [15]. These processes compromise the antithrombotic efficacy of LMWHs and might be implicated in the failure of thromboprophylaxis in patients undergoing MOS.

Measurement of anti-Xa plasma activity is still regarded as the only test for biological monitoring of LMWH treatment. Nevertheless, it has been shown that for patients receiving LMWH for thromboprophylaxis the concentration of anti-Xa activity in plasma is not well correlated with the clinical outcome, although this has not been the case for patients with acute coronary syndromes treated with LMWH [16–18]. Measurement of anti-Xa activity in plasma represents only one aspect of LMWHs' activity, since they interfere into several steps of blood coagulation. Dosage of anti-Xa activity is performed in platelet poor plasma. LMWHs' antithrombotic activity is partially inhibited by PF4 [14,18]. Global coagulation assays sensitive to LMWHs could probably better reflect their antithrombotic efficacy [19]. The analysis of the influence of LMWH treatment on the distinct phases of blood coagulation in clinical settings where the thrombotic risk is increased could be a breakthrough step towards the development of such assays.

The present study was designed to evaluate the alterations of thrombin generation in patients undergoing MOS, after receiving postoperative prophylaxis with fixed dose enoxaparin, as well as after thromboprophylaxis withdrawal. We also evaluated the variability of the individual response of the blood coagulation mechanism during the post-operative period.

Patients & Methods

Thirty one patients who underwent unilateral elective THR or TKR were included in the study. Patients were stratified according to surgical procedure: 15 patients whom underwent elective THR and received enoxaparin (4.000 anti-Xa IU once daily subcutaneous injections) for 30 days post-operatively and 16 patients whom underwent elective TKR and received the same dose of enoxaparin for 15 days post-operatively. Thromboprophylaxis administration followed the 9th ACCP recommendations [2,20–33]. The first subcutaneous injection was administered 8 hours after the end of surgery. Thigh length antiembolic stockings were pplied to all patients upon admission to the hospital and were applied for the full duration of thromboprophylaxis period.

Exclusion criteria were the presence of liver, renal or heart disease, cerebrovascular disease, known diabetes, peripheral artery disease, any known platelet or blood coagulation disorder, administration of any anticoagulant treatment (vitamin K antagonists, unfractionated heparin, LMWH, fondaparinux) and/or antiplatelet treatment (aspirin, clopidogrel, prasugrel) or NAIDS during the last 30 days before inclusion, contraindication for LMWH, cancer, pregnancy, estrogen therapy, and previous VTE episode.

All patients included in the study were tested for hereditary thrombophilia. They all had normal levels of antithrombin, protein C and S. Furthermore, all patients were tested and found to be negative for the factor V Leiden mutation and for the G20210A prothrombin gene mutation which has been previously reported to have a prevalence of 3.3% and 2.7%, respectively, among Greeks [34,35].

Thirty patients received spinal anesthesia with bupivacaine (8-12 mg) and fentanyl (20-25 μg) and only one patient received general anesthesia. The intraoperative monitoring was ECG/HR, non-invasive blood pressure (or invasive blood pressure when necessary), SPO_2, Temperature, Urine Output. Post-operative analgesia was achieved with paracetamol per os or intravenous and pethidine intramuscularly, or paracetamol and tramadol hydrochloride per os or intravenously.

The study was approved by institutional ethics committee and was done in conformity with the Declaration of Helsinki. All patients signed informed consent.

Healthy Individuals

The control group consisted of 15 age and sex matched healthy volunteers who had no personal history of thrombotic or haemorrhagic episodes and did not suffer from any other evident disease. Controls had normal prothrombin time and activated partial thromboplastin time. The healthy volunteers were not taking any medication for at least 1 month before blood sampling. The values obtained in the control group, were used to establish reference range for the assays.

Blood Samples

Blood samples were obtained by atraumatic puncture of the antecubital vein at the following time-points:

The day before surgery (D0); 7-hours after the end of the surgery and 1 hour before the administration of the first dose of enoxaparin (D1); at the 8th postoperative day (D8) (one hour before the next injection of enoxaparin); two days after the cessation of thromboprophylaxis: D32 for patients undergoing THR and D17 for patients undergoing TKR.

Platelet-poor-plasma (PPP) was obtained after double centrifugation of citrated whole blood for 20 minutes at 2000 *g*. Samples were aliquoted and frozen at -80 °C until assayed. All measurements were done in thawed plasma samples.

Thrombin Generation (TG) Assay

Thrombin generation in plasma was assessed using the Calibrated Automated Thrombogram assay (CAT®, Diagnostica Stago, France) according to manufacturers' instructions and according to the assay described by Hemker et al. [36], Briefly, 80 μl of PPP were added to 20 μl of PPP-reagent 5 pM® (Thrombinoscope b.v., Maastricht, Netherlands), a mixture of tissue factor (5pM final plasma concentration) and phospholipids (4 μM final concentration in plasma). Each patient's plasma was studied in duplicate. In a third well, PPP reagent 5pM® was replaced with the same volume of Thrombin Calibrator® (Thrombinoscope bv, Maastricht, Netherlands) to correct thrombin generation curves for substrate consumption and the inner filter fluorescence effects. Thrombin generation was triggered with a 20 μl solution containing $CaCl_2$ (16.7 mM final concentration) and the fluorogenic substrate Z-Gly-Gly-Arg-AMC (417pM final concentration). Fluorensence was measured using a Fluoroscan Ascent® fluorometer (ThermoLabsystems, Helsinki, Finland). Acquisition of thrombin generation parameters was done using the appropriate software (Calibrated Automated Thrombogram bv, Maastricht, The Netherlands).

Thrombogram parameters analysed were: the lag-time which describes the initiation phase of thrombin generation, the Peak concentration of generated thrombin and the time necessary to Peak (tt-Peak), the endogenous thrombin potential (ETP) that reflects the total amount of thrombin in it's active form in plasma, and the mean rate index (MRI) which reflects the velocity of the propagation phase of thrombin generation. The MRI is calculated by the formula MRI = Peak/(ttPeak-lag-time) [7].