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Impact of dabigatran on platelet function and fibrinolysis

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ABSTRACT

Background: We sought to evaluate the potential enhanced fibrinolytic and antiplatelet activity of dabigatran etexilate (DE) due to decreased thrombin levels in patients with stroke or transient ischemic attack and non-valvular atrial fibrillation (NVAF).
Methods: Consecutive patients with cerebrovascular diseases and NVAF that were treated with DE in a tertiary university hospital. Fibrinolysis and platelet function were assessed by thromboelastometry (ROTEM) and platelet function analyzer (PFA)-100, respectively, before and after treatment with DE. Conventional coagulation tests, endogenous thrombin potential (ETP) and hemoclot thrombin inhibitors (HTI), were also performed in order to detect any possible correlation between dabigatran plasma levels, its anticoagulant activity and the intensity of platelet dysfunction or fibrinolysis.
Results: A total of nineteen patients fulfilled our inclusion criteria (mean age 62.3 ± 7.2 years; 47% males; median $CHADS_2$-score: 3; interquartile range: 2–4). DE treatment was associated with a significant reduction of the lysis index (LI60) at 60 min ($p = 0.036$), and prolongation of the PFA-100 CEPI closure time ($p = 0.024$). After dabigatran treatment, abnormal PFA-100 results were obtained in two patients (11%, 95% CI: 2%–33%). DE levels (determined by HTI) were strongly inversely correlated (rho = –0.85; $p < 0.001$) with the area under the curve (AUC) values in ETP assay. No association was found between HTI and PFA-100 CEPI CT ($p = 0.64$), or LI60 measurements ($p = 0.60$).
Conclusions: Our findings indicate that DE might affect platelet function and fibrinolysis and highlight the potential role of ETP as an alternative option in DE monitoring. The intensity and clinical relevance of DE antiplatelet and fibrinolytic effects require further investigation.

1. Introduction

Oral direct inhibitors of both thrombin and factor Xa have now been developed and shown to be safe and effective for the primary and secondary preventions of stroke and systemic embolism in patients with non-valvular atrial fibrillation (NVAF) [1–3]. Dabigatran etexilate (DE) is a prodrug, which is hydrolyzed in the liver to the direct thrombin inhibitor dabigatran that is binding to the active site of thrombin [4], thereby attenuating fibrin formation, preventing thrombin-mediated feedback activation of factors V, VIII, and XI, and inhibiting thrombin-induced platelet activation [5]. Laboratory monitoring and dose adjustment for conventional anticoagulant treatment is not required due to its short half-life, wide therapeutic window and predictable anticoagulant response. There are, nevertheless, specific situations when clinical management requires knowledge of the intensity of anticoagulation, such as in patients with renal failure, at the extremes of body weight, in the case of DE complications (e.g. severe bleeding or thrombosis while on anticoagulant therapy), cases of potential overdose, in acute ischemic stroke patients presenting within the time window for intravenous thrombolysis and in the setting of scheduled or emergency surgery [6].

Our group has recently reported that compared to acenocoumarol, DE might affect platelet fibrinolysis and aggregation in NVAF patients treated with anticoagulation therapies for primary stroke prevention [7]. Particularly, in thromboelastometry (ROTEM®) analysis, the lysis index at 60 min (LI60) was significantly lower in patients receiving

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DE. This index is defined as the percentage of remaining clot stability in relation to the maximum clot firmness following the 60-min observation period after clotting time and indicates the speed of fibrinolysis. Furthermore, in light transmission aggregometry (LTA) patients on DE showed decreased aggregation compared to those on acenocoumarol that marginally did not reach the level of statistical significance. The influence of new oral anticoagulants on platelet function has been mainly investigated in vitro by using plasma spiked with these agents [8,9]. So, given the existence of a plausible underlying biological mechanism and the lack of data on the ex vivo impact of dabigatran on platelet function and fibrinolysis, we conducted a pilot study in order to evaluate this kind of effect in NVAF patients with ischemic stroke or transient ischemic attack (TIA) in the setting of secondary stroke prevention. We also investigated the potential correlation between dabigatran plasma levels (estimated by hemoclot thrombin inhibitors assay) and its anticoagulant activity (assessed using endogenous thrombin potential). Finally, we investigated the potential relationship between dabigatran plasma levels and the intensity of platelet dysfunction or fibrinolysis.

2. Methods

2.1. Study population

We evaluated consecutive patients with NVAF and history of stroke or TIA that were regularly followed up in the outpatient clinic of a tertiary care stroke center (Second Department of Neurology, Attikon University Hospital, Athens, Greece). Our previous short-term experience with the use of DE for secondary stroke prevention in NVAF patients has been recently reported [10]. All haemostatic parameters were analyzed before and after treatment with DE. Patients received the only dose of Pradaxa that was commercially available in Greece (110 mg per os bid) for stroke prevention (primary or secondary) during the study period (June 2013 to March 2014). More specifically, the higher dose of dabigatran (150 mg per os bid) was not commercially available in Greece during the study period. $CHADS_2$-score (congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, stroke/transient ischaemic attack) was estimated for all included patients. Individuals with platelet count $< 100 \times 10^9$ cells/L and active thrombosis or bleeding, patients under simultaneous anticoagulant and antiplatelet therapy, those with renal and/or hepatic and/or thyroid dysfunction, with malignancy or with chronic infectious or autoimmune diseases and patients with active infection or inability to swallow were excluded. We also excluded patients during the acute stage (first 14 days from the index event) of ischemic stroke and individuals who declined to sign informed consent documents. The study was performed in accordance with the Declaration of Helsinki and approved by the hospital's institutional review board.

2.2. Coagulation parameters

Before and 5 days after initiation of dabigatran treatment, the following laboratory exams were undertaken: complete blood count (CBC), partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen, D-dimers, platelet function analyzer 100 (PFA-100) and rotational thromboelastometry (ROTEM). Thrombin time (TT), endogenous thrombin potential (ETP), and hemoclot thrombin inhibitors (HTI) were performed, after a minimum of five days on dabigatran.

The last dose of antithrombotic medication was administered approximately 2 h before blood sampling. PFA-100, ROTEM analysis and conventional coagulation tests, were carried out on the same day, within 2 h of sampling. CBCs were performed on Sysmex XE-2100 analyzer (Roche, IL, USA). TT, aPTT, PT, fibrinogen and D-dimers, were all measured on BCS® XP System Hemostasis analyzer (Siemens Healthcare Diagnostics, Marburg, Germany). Pathromtin* SL (Siemens Healthcare Diagnostics, Marburg, Germany) was used to determine aPTT and the Thromborel® S Reagent was used for PT determination. TTs were assayed using BC Thrombin Reagent (Siemens Healthcare Diagnostics, Marburg, Germany). Plasma concentrations of fibrinogen were measured performing a modification of the Clauss method with Fibrinogen Multifibren* U reagent (Siemens Healthcare Diagnostics, Marburg, Germany) and D-dimers were assessed by the INNOVANCE D-Dimer assay (Siemens Healthcare Diagnostics, Marburg, Germany), a particle-enhanced immunoturbidimetric method.

Blood samples for HTI analysis were anticoagulated with 0.109 M trisodium citrate (9:1, v/v blood-anticoagulant) and immediately centrifuged at 2500 *g* for 20 min. For the ETP test, the blood specimen was centrifuged at 1500 *g* for 20 min, the supernatant was removed and was then centrifuged again. Plasma was snap frozen in small portions and stored at −20 °C until the assays were performed. Both tests were also performed on a BCS® XP System Hemostasis analyzer.

2.3. HTI

Dabigatran plasma levels were measured by HTI (Hyphen BioMed, Neuville-sur-Oise, France) is a sensitive diluted TT assay, which allows for quantitative measurement of dabigatran activity in plasma and has been used as the gold standard to assess its anticoagulant effect [11]. It is an in vitro diagnostic device intended to be used for the quantitative measurement of dabigatran in human citrated plasma, with a clotting method based on the inhibition of a constant and defined concentration of thrombin. For measuring DTI in plasma, first, the diluted plasma (1:8 to 1:20) was mixed with a normal pooled human plasma. Clotting was then initiated by adding a constant amount of highly purified human thrombin. The clotting time measured is considered to be directly related to the concentration of assayed DTI in the tested plasma. Intra-assay coefficient of variation for AUC was 7.88%.

2.4. ETP

INNOVANCE® ETP (Siemens Healthcare Diagnostics, Marburg, Germany) is a global hemostasis function test to assess the ETP of plasma samples. The incubation of plasma with phospholipids and activator and calcium ions leads to initiation and propagation of the coagulation processes, eventually resulting in the generation of thrombin. Thrombin generation (TG) and the subsequent inactivation were recorded by monitoring conversion of a specific slow reacting chromogenic substrate at a wavelength of 405 nm over time. The assay was performed using BCS® XP System Hemostasis.

The estimated parameters of the TG curve included the area under the curve (AUC), also referred to as ETP; the lag time (tlag) that describes the time from the initiation of the reaction until TG is being observed; the time to peak (tmax) which is the time from the initiation of the reaction until the maximum TG is being observed; and finally the maximum TG depicted by peak height (Cmax). The intra-assay coefficient of variation for AUC was 1.45%.

2.5. PFA-100

The platelet function analyzer (PFA)-100 was used to detect any antiplatelet effect caused by dabigatran due to decreased thrombin activity [12]. The PFA-100 system is a reliable, accessible and rapid method that detects platelet dysfunction by assessing the combination of both platelet adhesion and aggregation in whole blood [13,14]. The PFA-100 (Dade Behring, Marburg, Germany) measures the ability of platelets activated in a high-shear environment to occlude an aperture in a membrane treated with collagen and epinephrine (CEPI). The time taken for flow across the membrane to stop (closure time, CT) is recorded. For this test, whole blood collected in 3.8% trisodium citrate. 0.8 ml of the mixed whole blood was pipetted into the sample reservoir of 1 CEPI cartridge (prewarmed to room temperature) and then loaded into the PFA-100. The references ranges were 85–165 s as mentioned in the manufacturer's reagent instruction booklet. Abnormal platelet

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