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Thrombelastography detects dabigatran at therapeutic concentrations in vitro to the same extent as gold-standard tests

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ABSTRACT

Background/objectives: Dabigatran is an oral anticoagulant approved for treatment of non-valvular atrial fibrillation, deep venous thrombosis (DVT), pulmonary embolism and prevention of DVT following orthopedic surgery. Monitoring of the dabigatran level is essential in trauma and bleeding patients but the available plasma-based assays may not sufficiently display its hemostatic effect.

This study investigated the in vitro effect of different concentrations of dabigatran on whole blood thrombelastography (TEG) and its correlation to the specific but time-consuming plasma-based tests Hemoclot and Ecarin Clotting Time (ECT).

Method: Blood was collected from 8 healthy donors (two females, six males) with a median age of 46 years and it was spiked with dabigatran to a range of plasma concentrations (0, 50, 100, 200 and 400 ng/ml) covering the therapeutic level.

Results: Mean TEG R at 0 ng/ml = 5.963 min, 50 ng/ml = 7.425 min, 100 ng/ml = 8.425 min, 200 ng/ml = 9.775 min, 400 ng/ml = 11.813 min. A significant overall increase ($p = 0.001$) in TEG reaction time (R) was found across increasing dabigatran concentrations i.e. 0 vs 50 vs 100 vs 200 vs 400 ng/ml ($p < 0.000$ $p = 0.027$ $p = 0.026$, $p = 0.005$, respectively). TEG R correlated strongly with Hemoclot ($R^2 = 0.891$, $p < 0000$) and ECT ($R^2 = 0.914$, $p < 0.000$) and Hemoclot and ECT were strongly inter-correlated ($R^2 = 0.978$, $p < 0.000$).

Conclusion: The whole blood viscoelastic assay TEG R displayed linearity towards fixed concentrations of dabigatran and correlated strongly to the current gold-standard tests Hemoclot and ECT, for assessing dabigatran. TEG R is applicable as a rapid and precise whole blood monitoring test for dabigatran treated patients in the emergency setting.

1. Introduction

New oral anticoagulants have emerged on the market recently targeting patients suffering from atrial fibrillation (AF), deep venous thrombosis (DVT), pulmonary embolism (PE) and the prevention of DVT following orthopedic surgery. The Food and Drug Association (FDA) and the European Medicine Agency (EMA) approved dabigatran, a direct thrombin inhibitor, in 2012 [1]. As it has a predictable pharmacodynamic effect when taken orally it requires no routine monitoring and it is gaining ground on warfarin, the mainstay of oral anticoagulants since the 1950s. However, in the event of trauma, massive bleeding or emergency procedures, fast and reliable assessment and monitoring of the patients hemostasis is needed. Several tests for evaluating hemostasis are available, but in regards to dabigatran most are either not specific enough, too time-consuming in the clinical setting or not validated in clinical bleeding patients.

Activated partial thromboplastin time (aPTT) is regularly used to assess the degree of anticoagulation in patients treated with unfractionated heparin (UFH). aPTT can be used in dabigatran too, but it does not display linearity towards dabigatran concentrations; at concentrations above 100 ng/ml aPTT is prolonged unspecifically but not related to the actual dose of dabigatran [2] and though a high aPTT indicates supra therapeutic concentrations of dabigatran, the test does not reveal if the anticoagulant effect is of clinical relevance for e.g. bleeding risk [3].

The prothrombin time (PT) does not reflect dabigatran concentration [4]. Thrombin time (TT) reflects the concentration of dabigatran in a linear way though it has been shown to be too sensitive at high concentrations and should therefore primarily be used for excluding dabigatran as even low concentrations result in a prolongation of TT. [2,3] Furthermore it has not been validated in bleeding patients.

Ecarin Clotting Time (ECT) assesses dabigatran concentrations by dose-dependent inhibition a prothrombin–thrombin intermediate and is a reliable test, but not yet incorporated in the routine clinical setting.

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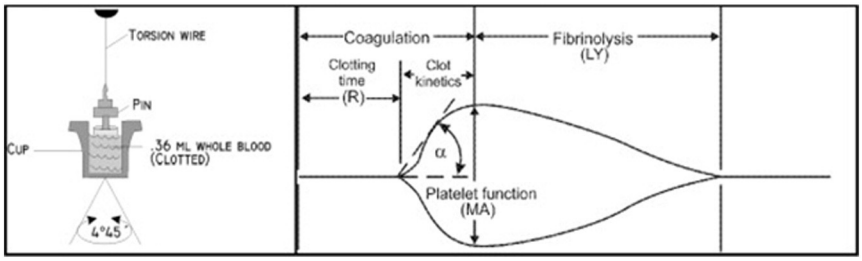

Fig. 1. Thrombelastographic analysis with measured parameters. Reaction time (R), alpha angle (α), maximal amplitude (MA) and lysis (LY).

The Hemoclot test [5] assesses dabigatran concentration by evaluating thrombin time on diluted plasma. It is probably the most reliable test when assessing the true concentration of dabigatran in plasma.

The above mentioned tests ECT and the Hemoclot are both not applicable to the emergency setting and lacks validation in patients with bleeding, trauma or critical illness.

In contrast, thrombelastography (Haemonetic Corp, Niles, Illinois, USA) (TEG), a viscoelastic, whole blood, hemostatic point-of-care (POC) test with results available within 14 min (Fig. 1), provides more relevant information on clinically relevant hemostatic changes than the plasma-based assays [6].

TEG is validated in the clinical setting for detection of coagulopathies [7], and has earned its place in the trauma setting and in the surgical theater by being an efficient and highly valued guide in transfusion therapy [8].

We [9] and others [10,11,12] have previously demonstrated that TEG is able to detect dabigatran anticoagulation in whole blood and the aim of the present study was to investigate the effect of increasing in vitro concentrations of dabigatran on functional hemostasis evaluated by TEG and furthermore to correlate the TEG results findings with the Hemoclot and the ECT tests. We hypothesized that TEG reaction time (R) would correlate dose-dependently to the concentration of dabigatran.

2. Materials and methods

2.1. Study participants

The study was conducted in accordance with the Declaration of Helsinki and approved by the regional ethics committee in Copenhagen, Denmark (protocol number: H-1-2013-65). Written information was available to each participant prior to trial entry. Blood was collected from 8 healthy Caucasian voluntary donors: six females and two males between the ages of 30–56 years. Exclusion criteria were intake of any kind of anticoagulant or antithrombotic medicine including intake of aspirin and non-steroid anti-inflammatory drugs 14 days before participating and pregnancy/breastfeeding.

The study was performed at Rigshospitalet, Capital Region Blood Bank, Copenhagen University Hospital, Denmark.

2.2. Blood sampling

Blood (9 mL, 3.2% citrate) was obtained by a smooth cubital venipuncture with a 21-gauge needle after employing minimal stasis.

2.3. Dabigatran

Dabigatran etexilate is a pro-drug that transforms into the active form, dabigatran, when hydrolyzed in plasma and in the liver. Dabigatran is a direct thrombin inhibitor that inhibits thrombin and thereby the formation of fibrin from fibrinogen. Furthermore it inhibits free and fibrin bound thrombin as well as thrombin induced platelet aggregation [13]. Since the present study investigated the in vitro effect of dabigatran, we used the active form of dabigatran, BIBR 953 ZW, kindly provided by Boehringer Ingelheim Pharma GmbH & Co.KG, Deutschland.

2.4. Study design

The standard dose of dabigatran is 150 mg BID. Patients displaying decreased renal function and patients aged ≥80 receive a lower dose of 110 mg dabigatran BID because 80% of the drug is excreted through the renal system. The standard dose of 150 mg BID results in a maximum concentration (Cmax) of 254 ± 70.5 ng/ml (mean ± SD) two hours after intake in healthy elderly subjects [14]. Plasma concentrations of 0, 50, 100, 200 and 400 ng/ml were chosen to mimic different concentrations of dabigatran that subjects would potentially present with after intake of the described dose of dabigatran [2].

2.5. Titration protocol

The active form of dabigatran etexilate, BIBR 953 ZW, was used and the powder was reconstituted in pure dimethyl sulfoxide (DMSO) and hydrogen chloride (HCL) according to instructions provided by Boehringer Ingelheim Pharma, Germany.

After reconstitution of the dabigatran etexilate powder BIBR 953 ZW a vial containing 26.56 μg/ml dabigatran was produced by diluting with DMSO. 12 μl of the this concentration was added to 1320 μl whole blood corresponding to a concentration of 400 ng/ml dabigatran in plasma (60% of the fluid phase of whole blood) The vial containing 26.56 μg/ml was diluted further by adding DMSO and vials corresponding to 200 ng/ml, 100 ng/ml and 50 ng/ml was produced. Whole blood with different concentrations of dabigatran was incubated for 15 min at 37 °C before analyzed or processed to plasma. All samples were diluted to the same extent (1%).

Plasma for freezing was produced by two centrifugations at 1800 g, 5 °C for 10 min and stored at −80 °C until Hemoclot and ECT.

2.6. Hemoclot

The Hemoclot Thrombin Inhibitor assay [5] (Hyphen BioMed, Neuville-sur-Oise, France [15]) is a thrombin time diluted assay mass concentration determination that calibrates dabigatran by a standard provided by Boehringer Ingelheim. It reports coagulation time in seconds, is calibrated by dabigatran and translates this to mass concentration of dabigatran in ng/ml by linear regression [5].

2.7. ECT

Ecarin Clotting Time quantitates direct thrombin inhibitors through thrombin generation and assesses dabigatran concentrations by dose-dependent inhibition of a prothrombin–thrombin intermediate. Prothrombin is converted to this intermediate by the snake venom ecarin [16,17] It is also reported in seconds and translated to a concentration in ng/ml by linear regression [16].

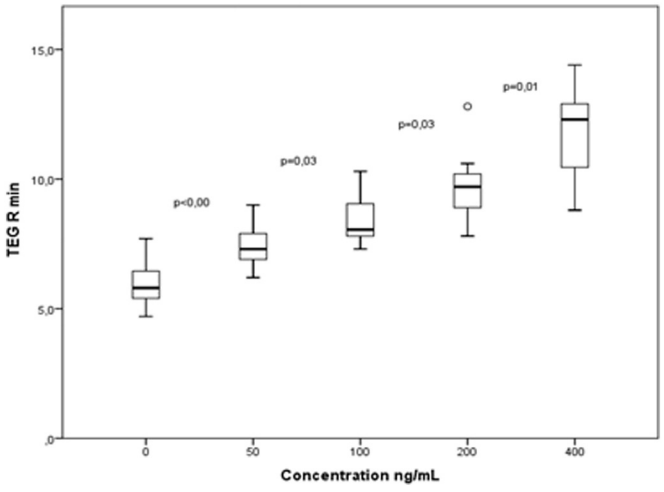

Fig. 2. TEG R at increasing concentrations. Figure displays TEG R in minutes with 95% interval of confidence and mean for each concentration. Concentrations are displayed in ng/ml and vary from 0 ng/ml up to 400 ng/ml covering the therapeutic area. Paired T-tests were conducted between concentrations (0–50 ng/ml, 50–100 ng/ml, 100–200 ng/ml and 200–400 ng/ml) and displayed between the boxes. All p-values were significant. Overall differences in the investigated hemostatic parameters were assessed by repeated measures ANOVA and presented as Wilks lambda p-value of $p = 0.001$.