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Monitoring of dabigatran anticoagulation and its reversal *in vitro* by thrombelastography

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Abstract

Background: dabigatran etexilate, a pro-drug of a direct thrombin inhibitor, was approved a few years ago for non-valvular atrial fibrillation and deep venous thrombosis. Rapid monitoring of the dabigatran level is essential in trauma and bleeding patients but the traditional plasma-based assays may not sufficiently display the effect. Furthermore, no antidote exists and reversal of the anticoagulant effect is impossible or difficult. The present study investigated the *in vitro* effect of dabigatran on whole blood thrombelastography (TEG) and its reversal by recombinant activated factor VII and prothrombin complex concentrate.

Methods: Blood was collected from 10 healthy donors and spiked *in vitro* with therapeutic doses of dabigatran to a plasma concentration of 200 ng/mL, followed by therapeutic doses of recombinant activated factor VII (corresponding to 100 µg/kg) and prothrombin complex concentrate (corresponding to 50 IE/kg) and evaluated by TEG.

Results: Compared to baseline, dabigatran changed all TEG parameters in a hypocoagulable direction corresponding to increased R time and time to maximum rate of thrombus generation, reduced angle, A5, A10, maximum amplitude and maximum rate of thrombin formation. Recombinant activated factor VII had a procoagulant effect on the majority of the investigated TEG parameters when added to dabigatran spiked samples. Prothrombin complex concentrate appeared not to have a procoagulant effect on TEG, even when the heparin content in the formulation was neutralized by heparinase.

Conclusions: TEG displays the presence of dabigatran in whole blood *in vitro* and the anticoagulant effect of dabigatran is partly reversed by spiking with recombinant activated factor VII.

Introduction

A new oral anticoagulant, dabigatran etexilate, a pro-drug of a direct thrombin inhibitor, was approved by the Food and Drug Association (FDA) and the European Medicine Agency (EMA) a few years ago for the treatment of patients with non-valvular atrial fibrillation (AF) and deep venous thrombosis (DVT)¹.

As dabigatran has a predictable pharmacodynamic effect when taken orally, no monitoring is recommended in routine clinical practice. However, in the clinical setting when patients undergo emergency procedures or experience bleeding episodes, monitoring of the anticoagulant effect is required. Monitoring dabigatran with traditional assays has however proven complicated and time consuming. Activated partial thromboplastin time (aPTT) shows a curvilinear dose-response to dabigatran with a steep increase at low concentrations. Above 100 ng/ml, the aPTT is invariably prolonged² and high aPTT indicates supra therapeutic levels although the anticoagulant effect tends to be underestimated³. A normal aPTT is likely to exclude the presence of therapeutic doses of dabigatran. The prothrombin time (PT) is insensitive to dabigatran⁴ whereas thrombin time (TT) shows a linear concentration curve to dabigatran. Finally, neither the aPTT nor the TT has been validated in a clinical relevant population of bleeding patients.

The results of TT however depends on the reagents used, with most TT assays being too sensitive^{2,4}. APTT has been recommended by many experts as a screening test of dabigatran anticoagulation but not as a test to determine the drug level⁵. With regard to reversing the drug effect, no antidote is clinically available at present and the means of reversal are few. An antidote was mentioned in Blood in 2013 measuring reversal of dabigatran anticoagulant activity in ex vivo in rats⁶.

Current strategies for reversal suggest discontinuation of the drug, supportive care, activated charcoal if ingested within one-hour, hemodialysis⁷ hemofiltration and charcoal hemoperfusion. In the presence of life-threatening bleeding, prothrombin complex concentrate (PCC), activated prothrombin complex concentrate (aPCC) or recombinant activated factor seven (rFVIIa), have been advocated⁸. The use and effect of fresh frozen plasma (FFP), PCC and rFVIIa are debated and no human studies have been conducted showing benefit of these treatments⁸. Thus, it is not known whether these drugs are useful for reversing the effects of dabigatran *in vivo*. The first study on the effect of PCC conducted on healthy humans showed that PCC does not reverse bleeding in patients treated with dabigatran.⁹ While this is also true for rFVIIa,¹⁰ both PCC and rFVIIa continue to be used in clinical settings when other resorts are futile⁸. Thrombelastography (TEG) is a viscoelastic, whole blood, hemostatic point-of-care (POC) test that delivers results within 15 minutes and is thus far more rapid than other tests. Its use has been implemented in trauma and many surgical settings to help guide transfusion therapy. Furthermore TEG is better correlated with clinical relevant bleeding conditions and is in many institutions preferred to and has replaced plasma-based assays¹¹.

Given the lack of data on the ability of TEG to detect dabigatran, the aim of the present study was to investigate the effect of dabigatran *in vitro* on functional hemostasis evaluated by whole blood TEG and furthermore to investigate the effect of *in vitro* reversal of dabigatran with PCC or rFVIIa. Since TEG is currently being used to guide transfusion strategy in bleeding patients, we hypothesized that this test would reflect dabigatran anticoagulation and change towards normalization upon reversal.

Materials and methods

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-4-2012-116). Written information was available to each participant prior to trial entry. Blood was collected from 10 healthy Caucasian donors: 8 women and 2 men between the ages of 21-65. Exclusion criteria were intake of any kind of anticoagulant or antithrombotic medicine including recent intake of aspirin and non-steroid anti-inflammatory drugs and pregnancy/breastfeeding.

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

Blood Sampling

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

Experimental protocol

The approved dose of dabigatran for atrial fibrillation is 150 mg two times daily resulting in a maximum concentration (C_{max}) of 254±70.5 ng/ml (mean±SD) in healthy elderly subjects¹². A plasma concentration of 200 ng/ml was chosen, to mimic the peak concentration of dabigatran in healthy elderly subjects². The active form of dabigatran, 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, Germany.

Reversal agents

The reversal agents used in the experiments were rFVIIa (NovoSeven, Novo Nordisk, Bagsvaerd, Denmark) and PCC (Octaplex, Octapharm, Stockholm, Sweden). The products were reconstituted according to instructions from the manufacturer. The concentrations of the reversal agents were calculated assuming a constant body weight of 70 kg with an average blood volume of 5 L. Octaplex is a nonactivated four-factor PCC derived from human plasma. 500U contains the procoagulation factors II (280-760 IE), VII (180-480 IE), IX (500 IE), and X (360-600 IE) as well as the natural anticoagulants Protein C (260-620 IE), Protein S (240-640 IE) and also heparin (100-250 IE). To neutralize the effect of heparin contained in Octaplex we supplemented our analyses with heparinase containing cups. Octaplex was added to citrated whole blood containing dabigatran to obtain a blood concentration of 0.7 IE/mL that corresponds to the blood concentration of a 70 kg patient given a therapeutic dose of 50 IE/kg. NovoSeven was added to citrated whole blood containing dabigatran to obtain a blood concentration of 1.4 µg/mL that corresponds to the blood concentration in a 70 kg patient given a therapeutic dose of 100 µg/kg.

Reversal protocol

9,6 µl dabigatran with a concentration of 13,200 µg/ml was added to 1060 µl whole blood, corresponding to a concentration of 200 ng/ml dabigatran in plasma (60 % of the fluid phase of whole blood).

15,5 µl FVIIa or 31 µl PCC corresponding to a therapeutic dose of 50 IE/kg or 100 µg/kg was added to the dabigatran spiked whole blood to reverse the effect of dabigatran.

Whole blood (baseline, BL), blood with dabigatran, blood with dabigatran and rFVIIa and blood with dabigatran and PCC were all diluted to the same extend (3,6%) to an end total volume of 1100 µl, incubated for 15 minutes at 37°C and analyzed by TEG.

Thrombelastography (TEG)

TEG[®] analyses were performed according to the manufacturer's recommendations on a 5000 Hemostasis Analyzer System (Haemonetics Corp., MA, US, TEG[®] Analytical Software 4.2.3). The coagulation process in the assay was initiated using kaolin as the activator. Normal cups; citrated kaolin (CK) and cups containing heparinase to neutralize heparin from Octaplex; citrated kaolin heparinase (CKH) were used. The variables recorded were [normal range reported by Haemonetics Corp.]: reaction time (R [3-8 min], rate of initial fibrin formation), angle (α [55-78 degrees], clot growth kinetics, reflecting the thrombin burst), amplitudes at 5 and 10 min after measuring the R-time (A5, A10, reflecting clot strength at predefined time points, see details for data extraction and extrapolation below), maximum amplitude (MA, clot strength [51-69 mm], reflecting maximum clot strength) and lysis after 30 min (Ly30 [0-8%], proportional reduction in the amplitude after MA, reflecting fibrinolysis), maximum rate of thrombus generation in mm/min (MRTG), time to maximum rate of thrombus generation (TMRTG) in minutes (see figure 1 for a visual description of variables). MRTG and TMRTG can be assessed in the "velocity" mode of the TEG software. The day-to-day CV% of TEGMA is < 7% in our laboratory¹³.

The TEG tracings were extracted from the TEG database for every individual CK and CKH sample and processed in Microsoft Excel to identify early amplitudes (A) at 5 and 10 minutes post R-time.

Statistics

Analyses were performed using the IBM SPSS 20 (IBM SPSS Statistics, Armonk, NY) statistical software. The TEG results are presented as medians with 25th and 75th inter-quartile range (IQR). Differences in the investigated hemostatic parameters from baseline after dabigatran exposure and its reversal were assessed by Friedman non-parametric repeated measures test and Wilcoxon signed rank post hoc test. p<0.05 was considered statistically significant.

Results

Data on TEG are presented in Table 1 and visualized in figure 2 and 3.

Influence of dabigatran on TEG

Compared to BL samples, dabigatran changed all TEG parameters (Figure 1) for both CK and CKH in a hypocoagulable direction corresponding to increased R time and TMRTG (Figure 1 and 3) and reduced angle, A5, A10, MA, MRTG; all $p < 0.05$ (Figure 1 and 3). Absolute values and relative changes are displayed in Table 1. The influence of dabigatran on the different TEG parameters were most pronounced for variables reflecting clot initiation and early clot build up (R, A5, A10, MRTG and TMRTG). Fibrinolysis assessed by LY30 was found to be minimal at baseline (CK: 0.7% (0.4-1.1) CKH: 0.9% (0.6-1.6)) and this parameter was omitted from further analyses.

Reversal of dabigatran by rFVIIa and PCC

Recombinant FVIIa had a procoagulant effect on the majority of the dabigatran spiked CK and CKH parameters, although rFVIIa did not restore these to baseline. Thus, rFVIIa added to dabigatran samples decreased R and TMRTG and increased A5 (for CK but not CKH), A10, angle and MA, though all remained relatively hypocoagulable compared to baseline (Figure 2).

In contrast to rFVIIa, PCC did not have a procoagulant effect on TEG but rather an anticoagulant effect, which in part reflected the heparin content in the PCC evidenced by the difference between relative changes in CK and CKH ($p < 0.05$ for all investigated parameters). Thus, by neutralizing the anticoagulant effect of heparin in PCC by applying the heparinased TEG cups, all parameters were significantly changed in a procoagulant direction (Figure 2A and 2B). To our surprise, CKH only reversed part of the anticoagulant effect even though the heparinase in the cups far exceeds the amount needed to neutralize the heparin content in PCC.

Discussion

The main findings in the present study were that dabigatran had a hypocoagulable effect on all the investigated TEG parameters and that rFVIIa partially reversed the dabigatran effect on TEG. PCC, however, did not reverse the anticoagulant effect of dabigatran but rather seemed to promote further hypocoagulability, which may be in part due to the heparin content in the PCC.

In accordance with another study¹⁴ TEG was capable of displaying the anticoagulant effect of the dabigatran concentration applied in the present study. Spiking plasma from healthy volunteers with the direct thrombin inhibitor dabigatran *in vitro* as a mean to mimic the effect on plasma-based coagulation assays *in vivo* has previously been described.¹⁵ Using whole blood viscoelastic tests in trauma and surgical settings to determine the degree of anticoagulation has recently been implemented¹⁶ and this provides a readily accessible, fast and low cost option for assessing hemostasis and reversal of treatment. TEG measures the different phases of clot formation (initiation, amplification, propagation) and we found that the clot initiation (reaction time) was prolonged according to the inhibitory effect of dabigatran on thrombin. The maximum amplitude signifying maximal strength of the formed clot and the angle were lower than at BL which emphasizes that thrombin inhibition also influences platelet function and the thrombin burst that takes place at the surface of activated platelets¹⁷.

Considering the partial reversal of dabigatran by rFVIIa, our results are in accordance with a rat tail bleeding time model in which rFVIIa was effective in correcting the coagulopathy.¹⁸ In a human healthy volunteer study however, rFVIIa failed to correct the suppressed thrombin generation induced by melagatran, another direct thrombin inhibitor¹⁰. Though the data on rFVIIa are few and inconclusive, rFVIIa is still recommended for live-threatening bleeding caused by dabigatran since no alternative hemostatic agent is currently available⁸.

In the present study, PCC did not reverse the effect of dabigatran but rather induced further anticoagulation, which to our surprise was only partially reversed by neutralizing the heparin content in PCC.

The remaining anticoagulant effects of PCC may be attributed to the content of the natural anticoagulant Protein S (240-640 IE per 500IE PCC) and Protein C (260-620 IE per 500IE PCC), which is also present in the tested PCC (Octaplex).

An *in vivo* study of Cofact, a four-factor prothrombin concentrate complex showed failure to reverse the effect of dabigatran¹⁹, whereas a study of Beriplex, another four-factor prothrombin concentrate complex, did reverse the coagulopathy caused by dabigatran monitored by volume of blood loss and time to hemostasis in a rabbit trauma model²⁰. Both of these products contain Protein C and Protein S.

In vivo studies demonstrating the efficacy of PCC are in a haemorrhagic scenario supported by tissue factor (TF) from subendothelial tissue beyond the supply from blood-borne cells. By extracting blood from the vessel, the source of TF originating from the subendothelium is excluded. Manufacturer's TF-based reagent ex-TEM has in previous studies²¹, due to high concentration, displayed inability to detect the effect of PCC in warfarin-treated patients in ROTEM® as well as giving homophiliacs a false normal thromboelastography profile. Therefore, monitoring PCC in viscoelastic haemostatic assays such as ROTEM/TEG are dependent on addition a specific concentration of TF in order to represent a coagulation similar to the *in vivo* milieu. With an insufficient supply of TF, it can be hypothesized that the anticoagulant agents tend to affect the coagulation to a greater extent than the procoagulants, hence the hypocoagulable results that maintained in this study. Supporting this theory, a study from 2007²² demonstrates the hypocoagulable effect of high dose PCC added to citrated whole blood. The study showed a predominant anticoagulant influence of PCC-derived heparin in ROTEM® as addition of protamine sulphate reversed the hypocoagulation.

The most important components of the Protein C pathway involve thrombin, thrombomodulin, the endothelial cell protein C receptor (EPCR), protein C and protein S. When Protein C is activated this irreversibly inactivates factors Va and VIIIa and thereby exerts its anticoagulant properties²³. Given that the endothelium is not present *in vitro*, the interaction between the components of this pathway and receptors residing at the endothelium is non-existing and this may in part explain why this paradox anticoagulant effect of PCC is observed *in vitro* but not *in vivo*²⁴. Furthermore, *in vivo*, most heparins are coupled to antithrombin (AT), which is bound to the endothelial glycocalyx, so the heparin effect of PCC becomes neutralized (and linked to the endothelium) when evaluated *in vivo*. Hence, the clinical importance of this PCC induced anticoagulation effect on the TEG parameters is not clear. rFVIIa is known to be prothrombotic²⁵ which PCC is not and one could speculate that in an *in vivo* system with all elements of hemostasis (endothelium, circulating cells; RBC, LEU, PLT etc. and pro- and anti-coagulation proteins) that this could have a beneficial thrombo-embolic protective effect.

The present study has some limitations. We included a small number of healthy volunteers that were only evaluated *in vitro*, leaving out e.g. the endothelium and its influence on hemostasis and the applied procoagulants. TEG also failed to monitor natural flow and shear stress effects on hemostasis. Several flow dependent automated microchip flow chamber techniques have been introduced and give other results upon correcting a dilutive coagulopathy with PCC than TEG²⁶.

Second, while we attempted to reproduce those doses and concentrations that might be seen *in vivo*, it is possible that the levels would be different (likely higher) in the population of patients that would be taking these medications (elderly with or without renal insufficiency). Finally, while our clinical groups have found good correlation with abnormal TEG values and life-threatening bleeding, disturbances of TEG values by dabigatran (and their partial reversal with VIIa) may not be clinically applicable.

In conclusion TEG displays the presence of dabigatran in whole blood *in vitro* and the anticoagulant effect of dabigatran can be partly reversed by spiking with rFVIIa whereas PCC did not seem to revert this when evaluated by TEG but the clinical importance of this needs to be further evaluated. Whether the latter reflects an *in vitro* phenomenon remains to be determined and so does the potential dose-response effect of dabigatran on TEG. Given the increasing number of patients receiving dabigatran and the relatively few studies conducted on the acute monitoring and reversal *in vivo*, studies investigating this are highly warranted.

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Figure 1. TEG Variables.

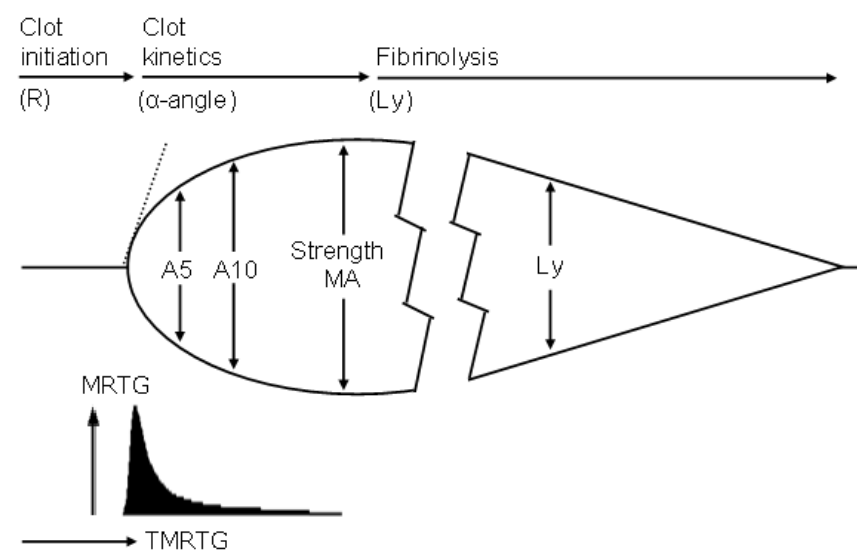


Table 1. Results

	Baseline	Dabigatran		Dabigatran + rFVIIa		Dabigatran + PCC		Friedman <i>p</i> (absolute)	
	Absolute	Absolute	Δ vs. baseline (%)	Absolute	Δ vs. baseline (%)	Absolute	Δ vs. baseline (%)	Dabi/rFVIIa ¹	Dabi/PCC ²
TEG									
CK R (min)	6.1 (5.8-6.2)	8.8 (7.9-9.1)*	+43 (40-48)	7.8 (7.1-8.4)*†‡	+30 (20-37)	20.1 (13.4-22.6)*†‡	+223 (135-276)*	<0.001	<0.001
CK angle (°)	61.4 (60.4-62.3)	52.1 (49-58.3)*	-17 (-20~-7)	56.4 (54.2-58.3)*†‡	-9 (-11~-7)	26.9 (24.6-32.7)*†‡	-55 (-60~-47)*	0.003	<0.001
CK A5 (mm)	37.3 (36.7-40.3)	28.3 (25.5-32.2)*	-26 (-32~-14)*	31.1 (29.8-32.2)*†‡	-19 (-20~-13)	11.9 (10.4-15.4)*†‡	-68 (-72~-61)*	0.001	<0.001
CK A10 (mm)	51.1 (50.4-54.6)	41.5 (37.1-44.9)*	-21 (-27~-14)*	44.1 (42.2-44.9)*†‡	-17 (-17~-12)	24.2 (22.7-31.2)*†‡	-51 (-55~-43)*	<0.001	<0.001
CK MA (mm)	60.8 (59.4-64)	53.6 (51-57.3)*	-13 (-17~-9)	56.4 (54.3-57.7)*†‡	-10 (-11~-8)	45.5 (44.6-45.9)*†‡	-26 (-28~-23)*	<0.001	<0.001
CK MRTG (mm/min)	9.7 (9.3-10.9)	7.5 (6.5-8.8)*	-27 (-35~-13)	7.9 (7.4-8.5)*†‡	-22 (-26~-12)	2.8 (2.7-3.9)*†‡	-69 (-72~-65)*	0.003	<0.001
CK TMRGT (min)	7.5 (7.1-7.6)	10.1 (9.5-10.6)*	+38 (31-42)	8.8 (8.1-9.4)*†‡	+17 (11-29)	25.8 (18.6-29.5)*†‡	+239 (145-312)*	<0.001	<0.001
CKH R (min)	6.3 (6-6.5)	9.2 (8.6-9.4)*	+46 (40-49)	8.2 (7.4-8.6)*†‡	+31 (25-33)	10.2 (9.1-11.1)*†‡	+63 (46-80)*	<0.001	<0.001
CKH angle (°)	60.4 (58.4-60.7)	51.6 (49-53.6)*	-17 (-21~-10)	57.0 (54.1-59.2)*†‡	-8 (-12~-2)	48.6 (46-52.5)*†‡	-18 (-24~-13)*	0.002	0.006
CKH A5 (mm)	35.4 (34.1-39.5)	28.1 (27-31.4)*	-23 (-26~-16)*	29.5 (28.9-32.4)*†‡	-17 (-20~-11)	27.1 (25.8-29.2)*†‡	-24 (-26~-19)*	0.003	0.002
CKH A10 (mm)	50.1 (48-53.7)	41.1 (39.5-45.4)*	-17 (-19~-14)*	42.3 (41.2-45.6)*†‡	-14 (-16~-10)	40.4 (39.4-42)*†‡	-20 (-21~-13)*	<0.001	0.001
CKH MA (mm)	61 (58.8-64.5)	53.9 (51.2-56.0)*	-12 (-15~-9)	55.5 (53.7-57.3)*†‡	-9 (-12~-7)	53.7 (52-55)*†‡	-12 (-15~-11)*	<0.001	0.001
CKH MRTG (mm/min)	8.9 (8.3-10.3)	7 (6.3-7.6)*	-22 (-33~-11)	8.1 (6.9-8.2) *†‡	-16 (-21~-3)	6.6 (6-7.1)*†‡	-27 (-31~-17)*	0.006	0.002
CKH TMRGT (min)	7.9 (7.3-8)	10.4 (9.9-11)*	+32 (26-37)	9.6 (8.6-9.7) *†‡	+22 (10-24)	11.7 (10.6-12.9)*†‡	+53 (27-65)*	<0.001	<0.001

TEG, thromboelastography; CK, citrated kaolin TEG; CKH, citrated kaolin with heparinase TEG; R, reaction time; A5, amplitude at R+5min; A10, amplitude at R+10min; MA, maximum amplitude; MRTG, maximum rate of thrombus generation; TMRGT, time to MRTG; TRAP, thrombin receptor activating peptide; AUC, area under the curve. Data shown as median (IQR) with absolute values and relative values compared to baseline. 1, Friedman' tests for the groups: Baseline, Dabigatran and Dabigatran + rFVIIa; 2, Friedman' test for the groups: Baseline, Dabigatran and Dabigatran + PCC. *, *p* < 0.05 vs baseline (Wilcoxon signed rank post hoc test); †, *p* < 0.05 versus Dabigatran; ‡, *p* < 0.05 for Dabigatran + PCC versus Dabigatran + rFVIIa; *, *p* < 0.05 for CK vs CKH.

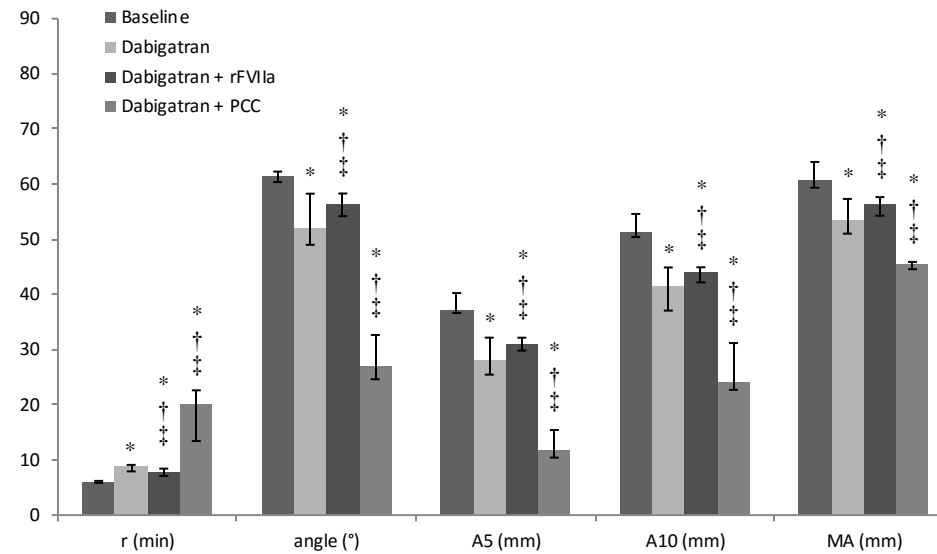
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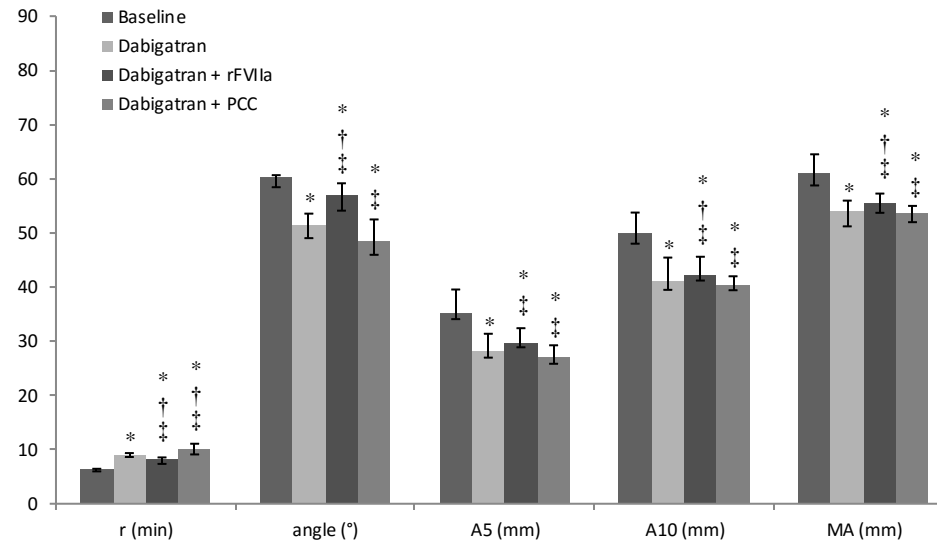
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Fig 2. TEG results

A. CK



B. CKH



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Reference List

- (1) Wallentin L, Yusuf S, Ezekowitz MD et al. Efficacy and safety of dabigatran compared with warfarin at different levels of international normalised ratio control for stroke prevention in atrial fibrillation: an analysis of the RE-LY trial. *Lancet* 2010 September 18;376(9745):975-83.
- (2) Lindahl TL, Baghaei F, Blixter IF et al. Effects of the oral, direct thrombin inhibitor dabigatran on five common coagulation assays. *Thromb Haemost* 2011 February;105(2):371-8.
- (3) van RJ, Stangier J, Haertter S et al. Dabigatran etexilate—a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. *Thromb Haemost* 2010 June;103(6):1116-27.
- (4) van RJ, Stangier J, Haertter S et al. Dabigatran etexilate—a novel, reversible, oral direct thrombin inhibitor: interpretation of coagulation assays and reversal of anticoagulant activity. *Thromb Haemost* 2010 June;103(6):1116-27.
- (5) Baglin T, Keeling D, Kitchen S. Effects on routine coagulation screens and assessment of anticoagulant intensity in patients taking oral dabigatran or rivaroxaban: guidance from the British Committee for Standards in Haematology. *Br J Haematol* 2012 November;159(4):427-9.
- (6) Schiele F, van RJ, Canada K et al. A specific antidote for dabigatran: functional and structural characterization. *Blood* 2013 May 2;121(18):3554-62.
- (7) Kaatz S, Kouides PA, Garcia DA et al. Guidance on the emergent reversal of oral thrombin and factor Xa inhibitors. *Am J Hematol* 2012 May;87 Suppl 1:S141-S145.
- (8) Makris M, van Veen JJ, Tait CR, Mumford AD, Laffan M. Guideline on the management of bleeding in patients on antithrombotic agents. *Br J Haematol* 2013 January;160(1):35-46.
- (9) Eerenberg ES, Kamphuisen PW, Sijpkens MK, Meijers JC, Buller HR, Levi M. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: a randomized, placebo-controlled, crossover study in healthy subjects. *Circulation* 2011 October 4;124(14):1573-9.
- (10) Wolzt M, Levi M, Sarich TC et al. Effect of recombinant factor VIIa on melagatran-induced inhibition of thrombin generation and platelet activation in healthy volunteers. *Thromb Haemost* 2004 June;91(6):1090-6.
- (11) Johansson PI. Goal-directed hemostatic resuscitation for massively bleeding patients: the Copenhagen concept. *Transfus Apher Sci* 2010 December;43(3):401-5.
- (12) Stangier J, Stahle H, Rathgen K, Roth W, Shakeri-Nejad K. Pharmacokinetics and pharmacodynamics of dabigatran etexilate, an oral direct thrombin inhibitor, are not affected by moderate hepatic impairment. *J Clin Pharmacol* 2008 December;48(12):1411-9.
- (13) Johansson PI, Svendsen MS, Salado J, Bochsén L, Kristensen AT. Investigation of the thrombin-generating capacity, evaluated by thrombogram, and clot formation evaluated by thrombelastography of platelets stored in the blood bank for up to 7 days. *Vox Sang* 2008 February;94(2):113-8.
- (14) Davis PK, Musunuru H, Walsh M, Mitra R, Ploplis V, Castellino FJ. The ex vivo reversibility of dabigatran-induced whole-blood coagulopathy as monitored by thromboelastography: mechanistic implications for clinical medicine. *Thromb Haemost* 2012 September;108(3):586-8.

- (15) Lindahl TL, Baghaei F, Blixter IF et al. Effects of the oral, direct thrombin inhibitor dabigatran on five common coagulation assays. *Thromb Haemost* 2011 February;105(2):371-8.
- (16) Cotton BA, McCarthy JJ, Holcomb JB. Acutely injured patients on dabigatran. *N Engl J Med* 2011 November 24;365(21):2039-40.
- (17) Monroe DM, Hoffman M, Roberts HR. Platelets and thrombin generation. *Arterioscler Thromb Vasc Biol* 2002 September 1;22(9):1381-9.
- (18) Van Ryn J. REVERSIBILITY OF THE ANTICOAGULANT EFFECT OF HIGH DOSES OF THE DIRECT THROMBIN INHIBITOR DABIGATRAN, BY RECOMBINANT FACTOR VIIA OR ACTIVATED PROTHROMBIN COMPLEX CONCENTRATE. D.Ruehl HPNHWW, editor. 2008.

Ref Type: Generic

- (19) Eerenberg ES, Kamphuisen PW, Sijpkens MK, Meijers JC, Buller HR, Levi M. Reversal of rivaroxaban and dabigatran by prothrombin complex concentrate: a randomized, placebo-controlled, crossover study in healthy subjects. *Circulation* 2011 October 4;124(14):1573-9.
- (20) Pragst I, Zeitler SH, Doerr B et al. Reversal of dabigatran anticoagulation by prothrombin complex concentrate (Beriplex P/N) in a rabbit model. *J Thromb Haemost* 2012 September;10(9):1841-8.
- (21) Sorensen B, Johansen P, Christiansen K, Woelke M, Ingerslev J. Whole blood coagulation thrombelastographic profiles employing minimal tissue factor activation. *J Thromb Haemost* 2003 March;1(3):551-8.
- (22) Takeyama M, Sakurai Y, Shima M et al. Heparin-induced inhibitory effects of a prothrombin complex concentrate on global tests of haemostasis. *Blood Coagul Fibrinolysis* 2007 January;18(1):1-7.
- (23) Chesebro BB, Rahn P, Carles M et al. Increase in activated protein C mediates acute traumatic coagulopathy in mice. *Shock* 2009 December;32(6):659-65.
- (24) Esmon CT. The protein C pathway. *Chest* 2003 September;124(3 Suppl):26S-32S.
- (25) Levi M, Levy JH, Andersen HF, Truloff D. Safety of recombinant activated factor VII in randomized clinical trials. *N Engl J Med* 2010 November 4;363(19):1791-800.
- (26) Schott U, Johansson PI. Bringing flow into haemostasis diagnostics. *Br J Anaesth* 2013 October 11.