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Enhanced fibrinogenolysis after dabigatran in normal healthy volunteers

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Abstract

Background: Dabigatran is an oral anticoagulant which differs from warfarin that it does not affect vitamin K dependent factors. It is grouped under the direct oral anticoagulant drug (DOAC) and acts as direct thrombin inhibitor. There is another DOAC which acts at different pathway and known as anti-factor X: a. Dabigatran as a direct thrombin inhibitor will theoretically not produce fibrinogenolysis. Itis therefore considered as safe from bleeding and does not need blood test for monitoring. Contradictory to this belief, there were reports that dabigatran produced severe bleeding in some cases.

Objectives: The aim of the study is to find out whether dabigatranas it is claimed to be safe from bleeding is a true direct thrombin inhibitor and does not produces fibrinogenolysis.

Materials and Methods: 15 normal volunteers were recruited in this study and venous blood samples were taken before dabigatran, 2 hours and 30 minutes after dabigatran, and 24 hours following its administration for the investigations of fibrinogen, thrombin time, and d-dimer.

Results and Discussion: Mean ± SEMoffibrinogenbefore dabigatrantends to reduce2 hours and 30 minutes after its administration 341.5 ± 17.9 and 306.9 ± 32.2 mg/dl respectively $p>0.05$ and becomes significant after 24 hours, 284.6± 19.6 mg/dl, $p<0.05$.d-dimer remains unchanged during the whole period, $p>0.05$ whereas thrombin time shows a classical prolongation of the test during dabigatran administration.

Conclusion: This study concludes that dabigatran produces enhanced fibrinogenolysis 24 hours after the administration but not fibrinolysis. In addition, thrombin time can not be used to detect the fibrinogenolysis. So, we recommend that fibrinogen should be measured for dabigatran monitoring.

Keywords: dabigatran, d-dimer, DOAC, fibrinogen, thrombin inhibitor, thrombin time

Introduction

Dabigatran is an oral anticoagulant known under the group of DOAC (Direct Oral Anticoagulant), and is claimed as direct thrombin inhibitor [1, 5]. This kind of DOAC is different from the classical oral anticoagulant warfarin which has an action to inhibit coagulation through inhibition of vitamin K dependent factors [6].The direct thrombin inhibitors work by inhibiting directly to thrombin (factor II: a coagulantion factor) or through inhibition of factor X: a coagulation factor [3, 5].Dabigatran which is known as the first DOAC introduced in the market and together with Ximelagatran are members thrombin inhibitors [1, 4]. There are other DOACS that act through different pathway, the example of those drugs are Rivaroxaban, Apixaban and others which are members of factor X: a inhibitors [1, 3]. In comparison with warfarin, the DOACS are claimed as safe and they do not need blood test to monitor such it is done with warfarin [7, 8, 9]. The classical test for warfarin monitor is prothrombin time (PT) which is expressed in International Normalized Ratio (INR) [7, 8].

Inspite of claims that DOACS do not need blood monitor and regarded as safe [8, 9, 16, 17], many reports published showed that dabigatran produced bleeding especially after several administration and sometimes could be severe [10, 14]. It is not clear why dabigatran a direct thrombin inhibitor which does not produce fibrinogenolysis may produce bleeding although it is given in its recommended dose [10, 15]. So, we are very interested in investigating why this contradictory phenomenon occurs. There are two possible causes of the bleeding; firstly dabigatran is not a true thrombin inhibitor, or there is another cause of bleeding by dabigatran outside of the coagulation pathway.

The aim of the study

This study is designed to find out if dabigatran as it is claimed as direct thrombin inhibitor does not really affect fibrinogen levels not only after a few hours of the administration but also several hours after later.

Materials and methods

15 normal subjects were recruited in the study after obtaining approval from the ethical committee of the Medical Faculty of the Universitas of Sumatera Utara/Hajj Adam Malik Hospital Medan with the number of 393/TGL/KEPK FK USU-RSUP HAM/2017, and the informed consent from the subjects. They have been given full information of the study design, the benefit for medical knowledge, and possible unpleasant effect during the study.

Subjects are resident's doctors, nurses and laboratory technologists, who have been screened and medically regarded as healthy.

The study starts with blood sample collection where blood sample was taken from median vein using 19G needlevenoject. The sequence of blood samplings are such as follows: before dabigatran administration, 2 hours and 30 minutes and 24 hours after of dabigatran administration. Dabigatran was given as 75 mg one tablet only. The blood taken was 1, 8 ml venous blood, and put into 0.2 ml of 3.2% sodium citrate, and mixed gently. The citrated blood was spun at 3000 x g for 30 minutes at 4^0C which yielded platelete poor plasma for the investigation of fibrinogen, thrombin time (undiluted and diluted), and d-dimer.

The test for fibrinogen uses clotting based assay acording to Klauss as described elsewhere [8, 15]. The reagent for fibrinogen test is from TEClot FIB Kit 10 from TECO Medical Instrument Production Trading GMBH, Germany. The assay used a standard curve whih plotted automatically in the automatic instrument of Coatron A4 TECO, Germany and the time to clot after the addition of the reagent into the sample is calculated against the standard curve. The result was expressed in mg/dl, and the reference range is 150-400 mg/dl.

Test for thrombin time was carried out using clotting assay where thrombin was added to the platelet poor plasma and the time until clot developed was measured [15, 17]. The reagent of thrombin used is from TEClot TT and measured in CoAtron A4 TECO machines from Teco, GMBH, Germany. The test before and 24 hours after dabigatran is done without diluting the plasma, while 2 hours and 30 minutes after dabigatran was performed both in diluted plasma and non-diluted plasma. The plasma was tested in dilution due to the reports that 2 hours and 30 minutes after administration, dabigatran reaches its peak concentration in vivo and non-diluted thrombin time is so prolonged which made it not appropriately interpreted and unreadable [8, 15, 17]. The dilution is 1:3 as recommended in some previous studies [17]. The result of thrombin time is expressed in second and the reference range is 11 seconds.

D-dimer assay is done based on immunoturbidimetric principle [18, 19] where the reagent containing antibody was obtain from Blue D-Dimer LC Kit TECO, Germany. The complex of d-dimer-anti-d-dimer antibody was obtained after incubation and the turbidity was measured based on light absorbance principle against known concentration at 460 nm. The result is expressed in ng/ml and the reference range is <500ng/ml.

Statistical analysis for significance test uses Wilcoxon test for non-parametric data, while the multivariate analysis for observing the trend is based on ANOVA or analysis of variants [20].

Result

Results of fibrinogen from 15 normal healthy volunteers before the administration of dabigatran, 2hours and 30 minutes after administration of dabigatran and 24 hours after the administration showed a trend of reduction of fibrinogen, however it does not achieve statistical significance (p<0,05). The statistical significance was seen 24 hours after dabigatran as compared to the level before administration. See table 1 and figure 1.

Table 1: Fibrinogen during 24 hours of 75 mg of Dabigatran

Mean ± SEM	
(mg/dl)	p value
Before	341,5 ± 17,9
2,5 hours	306,9 ± 32,2>0,05
24 hours	284,6 ± 19,6
< 0,05	

Anova test: p>0, 05 (n=15)

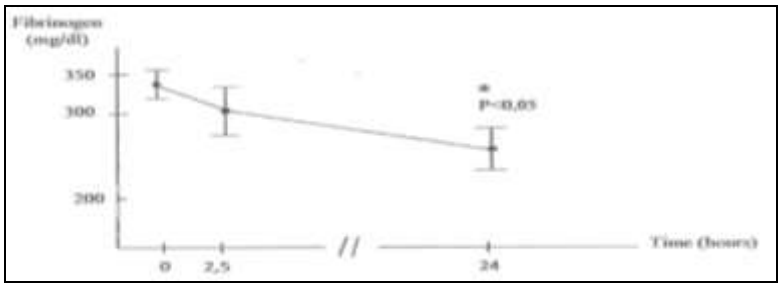

Fig 1: Fibrinogen levels 2.5 and 24 hours after 75mg of Dabigatran: wilcoxon test showed statistical significance 24 hours after administration.

In contrast to the fibrinogen, the d-dimer does not show any change during 24 hours of dabigatran (p>0,05) for both of 2 hours and 30 minutes as well as 24 hours after dabigatran compared to the initial level before administration of dabigatran, see table 2.

Table 2: D-dimer during 24 hours of75 mg of Dabigatran

Mean ± SEM	
(ng/ml)	p value
Before	96,0 ± 6,2
2,5 hours	76,4 ± 8,9> 0,05
24 hours	76,0 ± 10,0
< 0,05	

Significance test by Wilcoxon sum-rank test for non-parametric data, all are analyzed against"before dabigatran.

The result of thrombin time before and during administration dabigatran showed classical pattern of thrombin time prolongation [8, 15, 16].Where two and half hours the thrombin time is highly significantly prolonged (p<0,001).

Based on what other investigators use, this 2 hours and 30 minutes very prolonged thrombin time make the investigators need to dilute the plasma 1:3 dilution to shorten the result of thrombin time appropiately [17]. Dispite of this dilution the prolongation of diluted thrombin time showed similar significant to the non dilutional thrombin time (p<0,001).

However, the thrombin time is returning to near normal 24 hours after the administration of dabigatran although the p value is still very significance (p<0,001).

Table 3: Thrombin time during 24 hours of 75 mg Dabigatran

Mean ± SEM	
(second)	**p value**
Before	14,1 ± 0,3
2,5 hours	
Undiluted	89,1 ± 7,4< 0,001
Diluted	43,8 ± 10,7< 0,001
24 hours	18,1 ± 1,0< 0,001

Significance test by Wilcoxon sum-rank test for non-parametric data, all are analyzed against"before dabigatran".

Discussion

This study spesifically involves recruitment of normal healthy volunteers, where the subject do not show any evidence of thrombotic disorders. They have all been investigated medically for not having evidence of diseases. They are recruited from residents doctors, nurses, and laboratory technicians from our hospital and have been informed clearly about the aim of the study, the benefit of the study for this generation and also for future generation as a whole. The reason why normal subjects are choosen is to prove if dabigatran is really a true thrombin inhibitor [3, 4, 7]. If Dabigatran works as a partial thrombin inhibitor, it may partially cleaves fibrinogen into fibrin producing fibrin formation which shall hence be converted by factor XIII: a as activated by thrombin itself into x-linked fibrin. The increase of x-linked fibrin will physiologically digested by plasmin to produce increase in d-dimer [19]. In normal person when there is no evidence of thrombosis and dabigatran is given there should not be increase of D-dimer. If we use thrombotic patients in the study such as patients with DVT or stroke, we do not know if the increase of d-dimer is due to incomplete or partial inhibition of thrombin by dabigatran or due to the anticoagulation effect of dabigatran onto the already developed thrombus in the those thrombotic diseases.

One of the difficulties in this study is to recruit normal volunteers as some of them were afraid to have possible side effects such as bleeding, and this made this investigation as having high degree of difficulty and recruited small number of subjects. In reason to avoid the possibility of unpleasant effects during the study and for ethical reason we use dabigatran 75 mg given only one tablet in this study. This is far below the normal therapeutical dose which is 150 mg twice a day and given for a long duration of treatment [3, 5, 7, 8].

In this study it is clear that there is reduction of fibrinogen 2 hours and 30 minutes after dabigatran although it did not achieve statistical significance, however the reduction become significantly reduced 24 hours after the administration. This finding demonstrates that dabigatran produce fibrinogenolysis and it becomes so enhanced 24 hours later, therefore dabigatran is not a true direct thrombin inhibitor such as claimed by some investigator before.

Nonetheless, the result of d-dimer showed no increase during the whole period of study which means that dabigatran works as a thrombin inhibitor and it does not cleave enzymatically fibrinogen into fibrin. The evidence that dabigatrandid not produce increase in d-dimer shows that the enhanced fibrinogenolysis by dabigatran is not caused by partial inhibition or partial cleavage of fibrinogen into fibrin by thrombin. The pathway of dabigatran produces enhanced fibrinogenolysis needs further investigation. This phenomenom is very important as dabigatranin this study was given in a very low dose which was 75 mg one tablet only compared to 150 mg twice daily for longer period of time. It will not be surprising if more dramatic fibrinogenolysis happens when usual dose was given.

The result of thrombin time either undiluted or diluted as recommended for dabigatran monitoring which is sometimes advised for dabigatran monitoring in the elederly and done also in this study showed normal classical dabigatran effects but does not inform anything about fibrinogenolysis [8, 15, 16]. It is clear that thrombin time is not a sufficient test to avoid possibility of bleeding in dabigatran treatment. We hereby recommend that fibrinogen test should be tested on serial basis to all patients who received dabigatran. Before it is proven safe, the fibrinogen test should also be recommended as a monitor test to Ximelagatran another thrombin inhibitor.

Acknowledgment

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Statement of no conflict of interest

All authors hereby declare that there is no conflict of interest with other parties in this study.

Conclusion

This study demonstrate that dabigatran although a direct thrombin inhibitor but enhances fibrinogenolysis which is undetectable by thrombin time test. We hear by recommend that fibrinogen should be tested for every dabigatran treatment.

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