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Impact of Edoxaban on Thrombin-Dependent Platelet Aggregation

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Abstract

Edoxaban, a direct factor Xa inhibitor (FXa), is the fourth direct oral anticoagulant (DOAC) approved for clinical use. As the main adverse event is bleeding, it is relevant whether edoxaban has additional effects on platelet function. We aimed to assess in vitro aggregation in patients with atrial fibrillation (AF) receiving edoxaban. We evaluated 20 AF patients treated with edoxaban. We assessed light transmittance platelet aggregation (LTA) with 100 nmol/L γ-thrombin. The LTA was performed at 2 time-points. The thrombin-induced platelet aggregation was significantly lower 2 hours after edoxaban was taken compared to baseline measurement (27.25% $\pm$ 30.8% vs. 60.35% $\pm$ 33.3%). In addition, we also performed 16 subanalyses in order to identify the differences in the outcome of different comorbidities, age, dosage, liver and kidney function tests, and concomitant treatment. Results of the subgroup analyses were consistent with the findings of the main analysis; there was no apparent heterogeneity across the prespecified subgroups. The thrombin-induced platelet aggregation is reduced in non-valvular AF patients receiving edoxaban.

Keywords

atrial fibrillation, edoxaban, hemostasis, platelets, aggregation

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Introduction

Anticoagulation is a critical component in the management of venous thromboembolism (VTE) and atrial fibrillation (AF). Vitamin K antagonists (VKAs) have been the standard of care for the prevention and treatment of VTE, and stroke prevention in AF patients. However, VKAs are associated with a number of limitations that make achieving optimal conditions difficult and which consequently impact on patient care. These limitations highlight the need for new anticoagulants that are at least as effective but safer and more convenient to use. Knowledge of the various factors in the coagulation cascade and targeted drug design has led to the development of direct oral anticoagulants (DOACs).[1-3] Currently, 2 pharmacologically different groups of DOACs are used: factor Xa and factor IIa inhibitors.[4] Edoxaban is a selective factor Xa inhibitor. It is as effective as the VKAs with respect to the prevention of stroke or systemic embolism and is associated with significantly lower incidence of bleeding and death from cardiovascular causes.[5] From the patient perspective, edoxaban therapy has been shown to be more convenient to use than VKAs.[6,7]

Edoxaban inhibits free factor Xa without the need of antithrombin. This inhibition of factor Xa in the coagulation cascade leads to decreased thrombin generation, and therefore, a reduction in thrombus formation and progression.[8] The reduction in thrombin could also results in an indirect inhibition of platelet aggregation. In contemporary cardiology, we see an effort to combine DOACs with antiplatelet therapy. Therefore, it is relevant whether edoxaban has additional effects on platelet function. If so, it could affect the bleeding risk and influence the

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choice of treatment based on the patient risk profile. This effort is underlined by the fact that the effects of edoxaban in combination with a P2Y12 inhibitor in the setting of percutaneous coronary intervention are unexplored.[9] The social order to know the effect of edoxaban on platelet aggregation is therefore very strong.

The aim of the present study was to assess the effects of edoxaban on in vitro platelet aggregation in patients with non-valvular atrial fibrillation (NVAF).

Material and Methods

The local Ethical Committee of the Jessenius Faculty of Medicine in Martin approved this study (EK 1702/2015). All study participants agreed to participate in the project and signed a written informed consent in accordance with the Declaration of Helsinki.

Edoxaban was administrated once daily (7:00 AM). Blood samples were taken 24 hours after a previous drug dose administration (baseline, at 7:00 AM), followed by next blood sample after 2 hours (at 9:00 AM). To be sure that the drug was administered at the right and same time, we implemented the following measures. First, the drug was administered to the patients by a physician who was involved in this study. Secondly, the anti-Xa activity (ng/L) was assessed using edoxaban-calibrated anti-Xa chromogenic assays (BIOPHEN Heparin kit, BIOPHEN Edoxaban Plasma Calibrator, BIOPHEN Edoxaban Plasma Calibrator; Aniara Diagnostica, West Chester, OH). All patients on edoxaban therapy included in our study where hospitalized on the 1st Department of Internal Medicine during July and August 2018. We did not have any selection criteria. Concomitant treatment (e.g. beta blockers, proton-pump inhibitor . . .) was administered immediately after taking the morning dose of edoxaban.

Light transmission aggregometry (LTA) was performed using the international protocol for the laboratory investigation of platelet function.[10] We want to emphasize that testing was performed on patients without any antiplatelet or non-steroidal anti-inflammatory drugs (10 – 14 days before measurement) and with normal platelet count ($\geq 150 \times 10^9$/L). The antecubital venous blood was collected into tubes containing 3.2% buffered sodium citrate (anticoagulant-blood ratio 1:9) to assess platelet aggregation. Platelet aggregability was tested with platelet-rich plasma (PRP) using platelet aggregometry (PACKS-4 aggregometer, Helena Laboratories, USA). The platelet count in PRP was between 400 – 450 x 10^9/L. Blood samples were stimulated with human γ-thrombin in final concentration 100 nmol/L (Mybiosource Inc., San Diego, USA).

Data are presented as numbers with frequencies for categorical variables and means with standard deviations (± SD) for continuous variables. For comparison of the different groups, the closed-test-principle was used. An overall comparison was performed, followed by pairwise comparison if the results were significant. The p values less than 0.05 were considered statistically significant. Data were analyzed with SPSS 21.0.0.0 (SPSS Inc, Chicago, Illinois, USA).

Results

Table 1 presents full clinical baseline characteristics of the patients. Twenty patients with non-valvular AF were enrolled. The mean age was 71.0 ± 11.88 years (range 36-88 years),

Table 1. Clinical Baseline Characteristics.

Characteristics	Number of patients or value
Median age, years ± SD (range)	71.0 ± 11.88 (36-88)
<65 years	7
>65 years	13
Sex	
Male	11
Female	9
Duration of dabigatran treatment, median, days ± SD (range)	7.5 ± 16.02 (2-45)
≤7 days	10
>7 days	10
Indication	
Non-valvular atrial fibrillation	20
paroxysmal	13
persistent	3
permanent	4
Dose	
30 mg	4
60 mg	16
Risk factors	
Diabetes mellitus	7
Arterial hypertension	19
Renal disease	6
Dialysis, transplant, creatinine >2.26 mg/dL	0
Liver disease	1
Cerebral stroke/transient ischemic attack history	2
Coronary artery disease	
one vessel	2
two vessels	3
History of pulmonary embolism	3
Myocardial infraction history	4
BMI (kg/m^2)	
normal weight (18.5-24.9)	11
overweight (25.0-29.9)	8
obese (30.0 – 39.9)	1
Ischemic heart disease— Classification according to New York Heart Association	
I	8
II	11
III	1
CHA_2DS_2VASc* ± SD	3.65 ± 0.88
HAS-BLED^ score	2.1 ± 0.64
Platelets x10^9/L (range x10^9/L)	166 (140-309)
Median creatinine (μmol/L ± SD)	97.0 ± 18.8
Male	
normal (55-100)	5
pathological (>100)	6
Female	
normal (44-95)	5
pathological (>95)	4

(continued)

Table 1. (continued)

Characteristics	Number of patients or value
Median ALT (μkat/L ± SD) (ALT)	0.35 ± 0.29
normal (0.1-0.6)	17
pathological (>0.6)	3
Median AST (μkat/L ± SD) (AST)	0.4 ± 0.24
normal (0.1-0.6)	15
pathological (>0.6)	5
Median GMT (μkat/L ± SD)	0.56 ± 1.65
normal (0.07-0.63)	11
pathological (>0.63)	9
Drugs	
Beta blockers	18
Calcium channel blockers	9
Angiotensin converting enzyme blockers	13
Angiotensin II receptor antagonists	3
Antiplatelet drugs	0
Proton-pump inhibitor	10
Statins	6

*CHADS2-VASC score is presented as points on a scale of 1–9 (±SD)
^HAS/BLED score is presented as points on scale of 0 – 9 (±SD)
Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; GMT, gamma glutamyltransferase; SD, standard deviation.

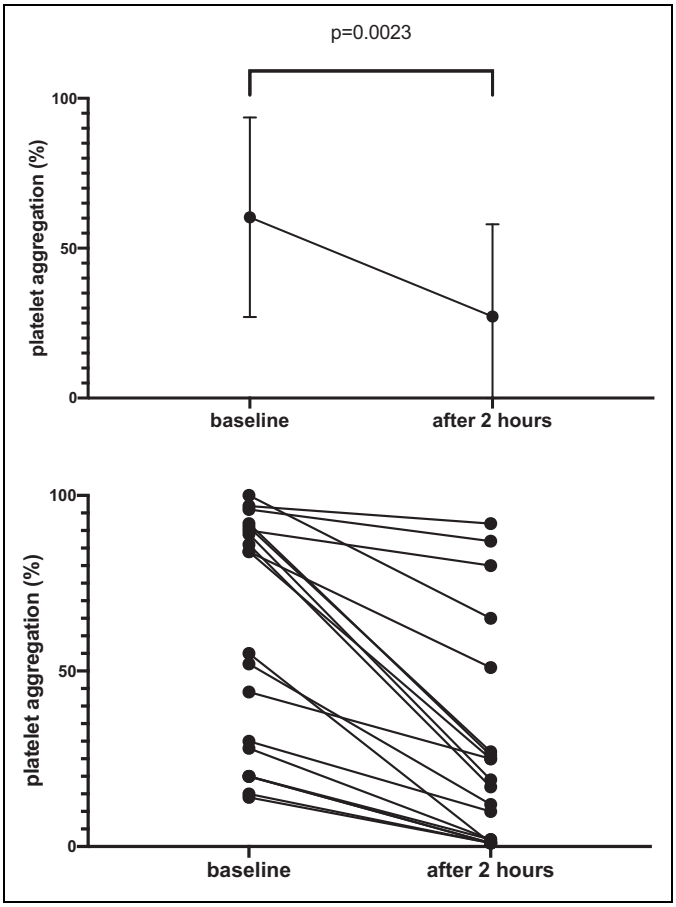

Figure 1. Results of thrombin induced platelet aggregation between groups.

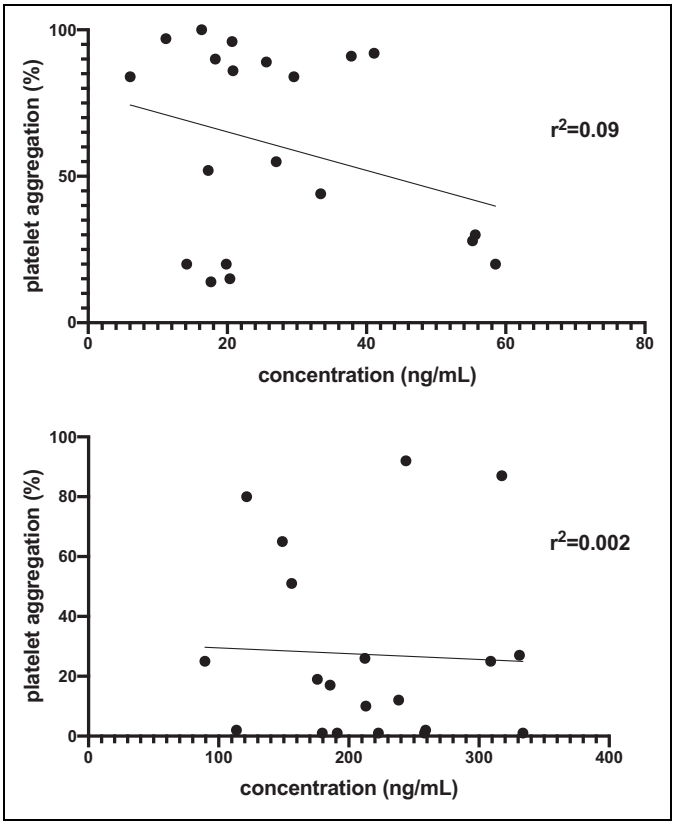

Figure 2. The dose-response curve for the plasma-diluted factor Xa time assay with edoxaban. Line represent the best-fit regressions for edoxaban.

9 patients were woman and the mean CHA_2DS_2VASc score was 3.65 ± 0.88. All patients began treatment with edoxaban as an initial anticoagulant treatment. Ten patients had an initial duration ≤ 7 days. Edoxaban doses were 30 mg (20%) or 60 mg (80%) once daily.

As shown in Figure 1, the thrombin-induced platelet aggregation was significantly lower 2 hours after edoxaban was taken compared to baseline measurement (27.25% ± 30.8% vs. 60.35% ± 33.3%, p<0.0023) in our study group.

The mean edoxaban concentration at baseline was 27.32 ± 15.8 ng/mL and 215.0 ± 72.17 ng/mL 2 hours after edoxaban was taken, respectively. The dose-response curve for the plasma-diluted factor Xa time assay with edoxaban had a correlation coefficient of $r^2 = 0.09$ for baseline and 0.002 for followed measurement (after 2 hours), see Figure 2.

We have done 16 subgroup analyses in order to determine the impact on aggregometer measurement, see Table 2. We did not find any significant difference between the groups.

Discussion

To our knowledge, this is the first prospective comprehensive study testing the effect of edoxaban on thrombin-induced platelet aggregation. This single-centre study assesses platelet aggregation in patients treated with edoxaban by LTA. The thrombin-induced platelet aggregation was significantly lower

Table 2. Subgroup Analysis.

Subgroup analysis	Thrombin induced platelet aggregation*	p value
≤65 years old vs. >65 years old	baseline: 60.4% ± 35.09% vs. 71.31% ± 27.7%	P = 0.14
	after 2 hours: 28.9% ± 33.4% vs. 31.7% ± 29.6%	P = 0.19
treatment duration: ≤7 days vs. >7 days	baseline: 64.7% ± 35.7% vs. 56.0% ± 31.9%	P = 0.57
	after 2 hours: 30.2% ± 29.1% vs. 24.3% ± 33.7%	P = 0.68
diabetes mellitus vs. without diabetes mellitus	baseline: 58.1% ± 39.6% vs. 56.9% ± 33.9%	P = 0.23
	after 2 hours: 28.3% ± 28.8% vs. 22.1% ± 31.8%	P = 0.35
with a history of renal disease vs without a history of renal disease	baseline: 52.7% ± 27.4% vs. 63.6% ± 35.9%	P = 0.51
	after 2 hours: 29.3% ± 17.4% vs. 20.6% ± 35.0%	P = 0.46
dose: 30 mg vs. 60 mg	baseline: 62.8% ± 23.2% vs. 67.3% ± 35.6%	P = 0.41
	after 2 hours: 32.3% ± 30.6% vs. 26.0% ± 31.7%	P = 0.73
normal weight vs. overweight and obese	baseline: 67.4% ± 33.5% vs. 51.8% ± 32.8%	P = 0.31
	after 2 hours: 27.4% ± 26.8% vs. 27.1% ± 36.7%	P = 0.97
normal creatinine level vs. pathological creatinine level	baseline: 66.9% ± 36.3% vs. 53.8% ± 30.4%	P = 0.39
	after 2 hours: 28.6% ± 38.0% vs. 25.9% ± 16.3%	P = 0.10
normal AST level vs. pathological AST level	baseline: 57.5% ± 31.0% vs. 49.0% ± 33.6%	P = 0.10
	after 2 hours: 24.2% ± 32.2% vs. 16.2% ± 11.6%	P = 0.34
normal ALT level vs. pathological ALT level	baseline: 55.4% ± 32.9% vs. 41.7% ± 20.2%	P = 0.17
	after 2 hours: 21.8% ± 31.2% vs. 11.3% ± 15.5%	P = 0.12
normal GMT level vs. pathological GMT level	baseline: 63.2% ± 30.3% vs. 54.7% ± 31.3%	P = 0.19
	after 2 hours: 32.5% ± 31.3% vs. 20.8% ± 30.6%	P = 0.41
beta blockers vs. no beta blockers	baseline: 44.4% ± 32.6% vs. 44.0% ± 5.7%	P = 0.11
	after 2 hours: 20.6% ± 21.2% vs. 12.0% ± 6.3%	P = 0.23
calcium channel blockers vs. no calcium channel blockers	baseline: 60.0% ± 34.0% vs. 60.6% ± 34.41%	P = 0.97
	after 2 hours: 33.0% ± 36.1% vs. 22.6 ± 26.5%	P = 0.46
angiotensin converting enzyme blockers vs. no angiotensin converting enzyme blockers	baseline: 63.9% ± 31.5% vs. 53.9% ± 37.9%	P = 0.54
	after 2 hours: 30.5% ± 31.6% vs. 21.3% ± 30.7%	P = 0.54
angiotensin II receptor antagonists vs. no angiotensin II receptor antagonists	baseline: 52.0% ± 37.0% vs. 61.8% ± 33.6%	P = 0.65
	after 2 hours: 20.7% ± 9.1% vs. 20.2% ± 32.44%	P = 0.32
proton-pump inhibitor vs. no proton-pump inhibitor	baseline: 52.2% ± 35.9% vs. 68.7% ± 29.8%	P = 0.27
	after 2 hours: 22.9% ± 33.2% vs. 31.6% ± 29.2%	P = 0.54
statins vs. no statins	baseline: 50.3% ± 38.3% vs. 64.4% ± 31.4%	P = 0.39
	after 2 hours: 32.5% ± 44.4% vs. 25.0% ± 24.6%	P = 0.63

*values are mean (± SD) for continuous variables.
Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; GMT, gamma glutamyltransferase; SD, standard deviation.

2 hours after taking edoxaban compared to baseline value. Based on our subgroup analysis, the results are independent of age, sex, dose, length of edoxaban administration, patient weight, selected biochemical parameters, and concomitant treatment.

It is likely that edoxaban affects platelets via 2 mechanisms. First is platelet activation. Although the central role of factor Xa as key propagator of coagulation that multiplies thrombin formation during primary haemostasis is extensively investigated, its impact on platelet activation is less well understood.[11,12] Rahman et al. have shown that low level of factor Xa enhanced intracellular phosphoinositide 3-kinase activity as well as inositol trisphosphate and diacylglycerol level and increased calcium signaling. This indicates a platelet activation.[13] The second mechanism is platelet aggregation. The mechanism of action of edoxaban is the inhibition of prothrombinase complex-bound and clot-associated factor Xa, resulting in reduction of thrombin burst during the propagation phase of the coagulation cascade.[14] Thrombin is not only a key protein in the cascade of fibrin clot formation but also a potent inducer of platelet aggregation.[15,16] Low thrombin level will result in reduced aggregation. Similar results were observed by TRAP-induced platelet aggregation in patients on dabigatran, rivaroxaban or apixaban treatment.[17,18] The study Vinholt et al. and the herby presented study confirmed the hypothesis that DOACs (dabigatran, rivaroxaban, apixaban and edoxaban) have an effect on platelet aggregation. In addition, Vinholt et al. reported that the receptor expression of GPIIb/IIIa, CD63, and P-selectin were reduced after dabigatran treatment.[19]

There were several limitations in our study, such as a small number of participants, which may have limited the possibility to detect drug effects on platelet function. Secondly, this study was not powered for clinical outcome. Therefore, it cannot be concluded that the combination of antiplatelet therapy and edoxaban is not safe. Thirdly, platelet aggregability is greatly affected by preanalytical issues; thus, interpretation of platelet hyperaggregability might be adversely influenced by prestudy factors.

Conclusion

The thrombin-induced platelet aggregation is reduced in non-valvular AF patients receiving edoxaban. This should be taken

into account in future studies of the combination of DOAC and antiplatelet therapy.

Declaration of Conflicting Interests

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