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% ± 30.8% vs. 60.35% ± 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). 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. The reduction in thrombin could also result 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. 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. 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 (≥ 150 × 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 × 10^9/L. Blood samples were stimulated with human γ-thrombin in final concentration 100 mmol/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).

| 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 infarction history | 4 |
| BMI (kg/m²) | |
| 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 |
| CHA2DS2-VASc* ± 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)
9 patients were women and the mean CHA2DS2-VASc score was 3.65 ± 0.88. All patients began treatment with edoxaban as an initial anticoagulant treatment. Ten patients had an initial duration of 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

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**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

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*SCHADS2-VASC score is presented as points on a scale of 1–9 (±SD)*

**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.

**Abbreviations:** ALT, alanine transaminase; AST, aspartate transaminase; GMT, gamma glutamyltransferase; SD, standard deviation.
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 AST level vs. pathological AST 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 ALT level vs. pathological ALT 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 GMT level vs. pathological GMT 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 weight vs. overweight and obese                      | 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% ± 34.4% | P = 0.97 |
|                                                             | after 2 hours: 33.0% ± 36.1% vs. 22.6% ± 26.6% | P = 0.46 |
| angiotensin converting enzyme blockers vs. no angiotensin   | baseline: 63.9% ± 31.5% vs. 53.9% ± 37.9% | P = 0.54 |
| converting enzyme blockers                                  | after 2 hours: 30.5% ± 31.6% vs. 21.3% ± 30.7% | P = 0.54 |
| angiotensin II receptor antagonists vs. no angiotensin II    | baseline: 52.0% ± 37.0% vs. 61.8% ± 33.6% | P = 0.65 |
| receptor antagonists                                         | after 2 hours: 20.7% ± 9.1% vs. 20.2% ± 32.4% | 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. Rahman et al. have shown that low level of factor Xa enhances intracellular phosphoinositide 3-kinase activity as well as inositol trisphosphate and diacylglycerol level and increased calcium signaling. This indicates a platelet activation. 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. Thrombin is not only a key protein in the cascade of fibrin clot formation but also a potent inducer of platelet aggregation. 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. The study Vinholt et al. and the herby presented study confirmed the hypothesis that DOACs (dabigatran, rivaroxaban 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.

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
Declaration of Conflicting Interests
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