

# The effect of enoxaparin on in vitro stimulated platelet aggregation by elective percutaneous coronary intervention patients

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## Abstract

**Aim:** The aim of the present study was to investigate the effects of enoxaparin on in vitro stimulated platelet aggregation by elective percutaneous coronary intervention patients.

**Method:** Twenty-two patients that scheduled for elective percutaneous coronary angioplasty (PTCA) were enrolled in the present study. The patients who had not been taking any antiaggregant agent other than aspirin and normal platelet account received enoxaparin (1 mg/kg IV bolus) as anticoagulant agent during PTCA. Two blood samples were obtained for every patient via femoral arterial sheath during the intervention before and 10 minutes after enoxaparin administration and stimulated platelet aggregation responses are investigated.

**Results:** The decrease in platelet aggregation responses to adenosine diphosphate (ADP), collagen and epinephrine before and after enoxaparin administration were statistically significant ( $p < 0.05$ ). The decrease in platelet aggregation response to ristocetin before and after enoxaparin was not statistically significant ( $p > 0.05$ ).

**Conclusion:** Enoxaparin may reduce platelet aggregation in elective PTCA patients pretreated with aspirin only. With the knowledge of the importance of the platelet inhibition, choice of the anticoagulant agent during PTCA may be beneficial.

**Keywords:** Enoxaparin, stimulated platelet aggregation, elective PTCA

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## Introduction

Subcutaneous (SC) enoxaparin has been shown to be better choice than unfractionated heparin (UFH) in the medical treatment of unstable angina (UA) and non-ST- elevation myocardial infarction (NSTEMI).<sup>(1-4)</sup> Combined analysis of two studies, the Efficacy and Safety of Subcutaneous Enoxaparin in Non-Q-Wave Coronary Events (ESSENCE) and Thrombolysis In Myocardial Infarction (TIMI) 11B, showed that enoxaparin reduced the risk of death and MI by 20% at 43 days without any significant increase of major hemorrhage.<sup>(5)</sup> ESSENCE and TIMI 11B trials demonstrated superiority of enoxaparin over UFH at 43 days for end points death or myocardial infarction (MI) and death or MI or urgent revascularization in patients who did not undergo percutaneous coronary intervention (PCI).<sup>(5)</sup>

In this analysis, excluding events that occurred before PCI, there was also superiority of enoxaparin treatment over UFH measured at 1 year for both end points in patients who underwent PCI.<sup>(5)</sup> Several studies have shown good outcomes with SC enoxaparin anticoagulation of patients with UA/NSTEMI underwent PCI on SC enoxaparin treatment.<sup>(6-8)</sup> Intravenous (IV) low-molecular-weight heparins (LMWH) in elective PCI have been evaluated and shown to be safe and effective in various registries.<sup>(3,9-11)</sup>

There are several studies that measure platelet reactivity in stable PCI patients using different methods.<sup>(12-14)</sup> The aim of the present study was to investigate the effect of enoxaparin on in vitro stimulated platelet aggregation by using % aggregation test to certain agonists in elective coronary intervention patients and the clinical significance of aggregation response to enoxaparin.

## Materials and Methods

### Study Population

Study population comprised patients older than 30 years with stable angina pectoris and documented ischemia (positive treadmill exercise test or positive myocardial perfusion scan for ischemia), who underwent coronary angiography and scheduled for elective PCI.

### Exclusion criteria were:

1. unstable state or acute coronary syndrome,
2. stable angina with an identified precipitating factor (e.g. severe anemia, heart failure, tachyarrhythmia, thyrotoxicosis, severe uncontrolled hypertension),
3. myocardial infarction or PCI in previous month or coronary bypass surgery in 2 months,
4. treatment with UFH or LMWH >24 hours of any cause before enrollment,
5. treatment with any other anti-platelet agent than aspirin (e.g. clopidogrel, ticlopidin, dipyridamol),
6. refusal of patient

22 patients, 16 males and 6 females were enrolled to the study. Mean age was  $56.8 \pm 1.6$  years. Mean age for female patients was  $57.4 \pm 2.1$  and for male patients  $56.7 \pm 1.4$ . Seventeen patients (14 of males and 3 of females) were smoker. Only 3 patients (2 of males and 1 of females) had diabetes mellitus and none were under insulin treatment. Fifteen patients (11 of males and 4 of females) had hypertension and were under treatment. Seventeen patients (12 of males and 5 of females) were under statin treatment since their coronary angiography procedures.

Concomitant medication was similar in male and female patients. All of patients received daily aspirin (ASA) > 100 mg more than 7 days before procedure. Fifteen of males and 5 of females were taking beta-blockers. Fourteen of males and 5 of females were using nitrates. All hypertensive patients were taking ACE-inhibitors and all of hyperlipidemic patients were taking statins.

### Blood Collection

Immediately after femoral sheath replacement the first blood sample was drawn through the sheath to determine the basal (before anticoagulant) platelet aggregation response. Enoxaparin was given 1 mg/kg (100IU/kg) IV bolus after PTCA-wire crossed the lesion as a single dose. Ten minutes after the administration of enoxaparin the second blood sample was drawn through the sheath.

### *In vitro Platelet Aggregation Measurement*

Immediately after blood collection, platelet aggregation measurements were made. All blood samples were studied into 2 hours after their collection. In vitro platelet functions were evaluated in the hematology laboratory with aggregometer. Stimuli were adenosine diphosphate (ADP), collagen, epinephrine and ristocetin. PAP-4CD Bio-Data was used for platelet aggregation.

### *Statistical Analysis*

A student-paired t-test was applied to assess the differences between pre- and post-enoxaparin platelet aggregation responses. Results are expressed as mean  $\pm$  standard error (SE). The level of significance was set at  $p < 0.05$ . SPSS 10.0 software was used for the statistical analysis.

The alteration in the platelet aggregation response with certain stimuli before and after enoxaparin is investigated in order to explore if enoxaparin has an additional antiaggregant effect.

### **Results**

Baseline clinical characteristics of study population and concomitant medications are described in **Table 1 and 2**. Baseline platelet counts were within normal limits in the study population and were unchanged after the intravenous administration of enoxaparin ( $212 \pm 48 \times 10^6$  before and  $210 \pm 42 \times 10^6$  after enoxaparin). Not any major bleeding complication occurred. None of patients had required blood transfusion. Only 3 of patients had small hematoma on the femoral access site. In this study not any in-hospital complication (such as ischemic complications or infection) was experienced.

Stimulated platelet aggregation responses before and after enoxaparin are summarized in **Table 3**. Values are given as mean  $\pm$  SE of % aggregation. Platelet aggregation responses to ADP were  $36.2 \pm 4.6$  before and  $29.3 \pm 3.4$  after enoxaparin ( $p=0.005$ ) and to epinephrine  $41.4 \pm 5.3$  before and  $30.5 \pm 4.7$  after enoxaparin ( $p=0.02$ ). The difference was statistically significant. Platelet aggregation responses to collagen were  $57.8 \pm 5.0$  before and

**Table 1. Patient Demographics**

Demographics	Male (N=16)	Female (N=6)
Age	56.7 $\pm$ 1.4	57.4 $\pm$ 2.1
Smoker	14	3
Diabetes Mellitus	2	1
Hypertension	11	4
Hyperlipidemia	12	5

*N* indicates number of patients. Age is given mean  $\pm$  SE in years.

**Table 2. Concomitant medications of the study patients**

Medication	Male (N=16)	Female (N=6)
ASA>7 days	16	6
$\beta$ -blocker	15	5
Nitrate	14	5
ACE-I	11	4
Statin	12	5

*N* indicates number of patients.

**Table 3. Stimulated Platelet Aggregation Responses**

Stimuli	Before Enoxaparin (N=22)	After Enoxaparin (N=22)	P value
Adenosine diphosphate (ADP)	36.2 $\pm$ 4.6	29.3 $\pm$ 3.4	0.005
Collagen	57.8 $\pm$ 5.0	45.7 $\pm$ 6.0	0.05
Epinephrine	41.4 $\pm$ 5.3	30.5 $\pm$ 4.7	0.02
Ristocetin	75.0 $\pm$ 3.0	67.0 $\pm$ 4.3	0.07

*N* indicates number of patients. Data are given mean  $\pm$  SE of % aggregation. A value of  $P < 0.05$  is considered statistically significant.

45.7 ± 6.0 after enoxaparin (p=0.05); to ristocetin 75.0 ± 3.0 before and 67.0 ± 4.3 after enoxaparin (p=0.07).

## Discussion

Despite of new tools, new drugs and new techniques, clot formation remains still as an important issue in interventional cardiology. The UFH has been the primary anticoagulant agent for more than 30 years, but the optimal dosage, the monitoring of anticoagulation level and the interaction between UFH and platelets remain controversial.<sup>(15-17)</sup> LMWHs offer a stable and predictable anticoagulant response and do not need for coagulation monitoring.<sup>(18)</sup>

Enoxaparin has shown its superiority to UFH in the medical treatment of UA and NSTEMI.<sup>(1,2)</sup> There is also a meta-analysis revealing a better evolution in ST-segment elevation acute myocardial infarction patients, who received enoxaparin instead of UFH as an adjunctive therapy to the thrombolytic regimen.<sup>(4)</sup> Several studies have also shown good results with enoxaparin on UA/NSTEMI patients who underwent PCI.<sup>(6-8)</sup>

There are some potential mechanisms, which may explain beneficial effects of enoxaparin.<sup>(18)</sup> Because of the molecular features enoxaparin may permit more suppression of thrombin generation than UFH with a higher antifactor Xa: antifactor IIa (thrombin) ratio (3.8:1).<sup>(18)</sup> Enoxaparin has a prolonged antifactor Xa activity and higher antifactor IIa activity than UFH because of better bioavailability, is less sensitive to the inhibitory effects of platelet factor 4, may release the tissue factor pathway inhibitor with greater capacity, has a lower tendency to increase activation and aggregation of platelets and shows potential antiplatelet effects while suppressing von Willebrand factor greater degree.<sup>(18, 19-28)</sup>

The aim of this study was to investigate the aggregation response of stable patients to enoxaparin via platelet functions beyond its anticoagulation activity. To the best of our knowledge, this is the first study that compared parameters of platelet aggregation before and after administration of enoxaparin during elective PCI in patients pretreated only with aspirin. All unstable patients were excluded to avoid the interaction between activated aggregation and coagulation cas-

cade and platelet aggregation response. In the present study, enoxaparin showed a slight decrease of % aggregation on platelet aggregation responses stimulated with collagen and ristocetin but it was statistically not significant. Enoxaparin significantly decreased platelet aggregation responses stimulated with ADP and with epinephrine in elective PCI patients. None of our patients experienced any ischemic or hemorrhagic complications neither in 72 hours nor in 30 days in follow-up.

Platelets play a key role in the development of thrombotic events during and after PCI.<sup>(29-31)</sup> There are multiple pathways of platelet activation and aggregation. Thrombin is the most potent agent, which activates platelets in subnanomolar concentrations via protease-activated receptors (PARs).<sup>(31,32,33)</sup> PAR activation results ADP release from dense granules, which acts in an autocrine way on the platelet ADP receptors.<sup>(31,34)</sup>

This endogenous ADP release may be reduced via enoxaparin resulting in significant decrease of ADP induced platelet aggregation. In a previous study Xiao et al. investigated platelet aggregation response to enoxaparin in patients taking only aspirin with UA and found a modest but not statistically significant increase in platelet aggregation with enoxaparin.<sup>(25)</sup> The conflict may be related to the study population. While UA patients have been included to the study of Xiao et al, all UA patients were excluded from the present study.<sup>(25)</sup> In another study, Aggarwal et al. have found that anticoagulation with enoxaparin during hemodialysis is associated with less platelet reactivity in a different study population.<sup>(35)</sup>

The limitations of the present study were small number of patients and lack of unstable patients. In acute coronary syndromes and unstable patients, especially by the site of thrombus, antiaggregant effect of enoxaparin may be more critical, but further studies are needed. In conclusion, enoxaparin may reduce platelet aggregation in elective PCI patients treated with aspirin only. With the knowledge of the stronger platelet inhibition, the lower the incidence of ischemic complications,<sup>(36-39)</sup> choice of the anticoagulant agent or additional antiaggregant agents during PCI may be beneficial.

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