Supplementary Material

In Vitro Studies of the Anti-Platelet Action of Enoxaparin

Materials: Blood was taken into tubes containing 3.13% (wt/vol) tri-sodium citrate dihydrate. Fluo-4 AM, probenecid (water soluble) and CaCl₂ were from Fisher Scientific (Loughborough, UK). Thrombin was from Sigma-Aldrich (Gillingham, UK). PAR1 agonist peptide (SFLLRNPNDKYEPF) was synthesized by Almac Group Ltd (Craigavon, UK). Enoxaparin (Clexane) was from Sanofi-Aventis (Guildford, UK). Anti-thrombin 3 (ATIII) was from Haematologic Technologies Inc (Vermont, United States). ATIII control was 50% glycerol/water (vol/vol). HEPES Tyrodes buffer was 129 mmol/L NaCl, 8.9 mmol/L NaHCO₃, 2.8 mmol/L KCl, 0.8 mmol/L KH₂PO₄, 5.6 mmol/L dextrose and 10 mmol/L HEPES.

Methods: Blood was obtained from healthy volunteers who had not taken any anti-platelet medication in the previous 10 days and anti-coagulated with 3.13% (wt/vol) tri-sodium citrate at a ratio of 1:9. Volunteers gave written informed consent and all studies were performed with local ethics board approval. Blood samples were centrifuged at 200 × g to produce platelet-rich plasma (PRP). PRP was labelled with fluo-4 AM (3 μmol/L) in the presence of probenecid (2.5 mmol/L) for 30 minutes at 37°C. Fluo-4 AM labelled PRP was pre-incubated with enoxaparin (0.25–2 anti-Xa U/mL) for 30 minutes. Small aliquots of labelled PRP (2 μL) were diluted 1:250 with HEPES-Tyrodes buffer containing a physiological concentration of ATIII (0.125 mg/mL) or control, 1 mmol/L calcium chloride and enoxaparin at the concentrations indicated in the ‘Results’ section. Baseline fluorescence was measured for 10 seconds using Becton Dickinson LSRII flow cytometer (Oxford, UK). Thrombin (0.5 U/mL) was then added directly to the vial and fluorescence was monitored for a further 2 minutes. Area under the curve for increase in median fluorescence was determined. Statistical analysis was performed using the GraphPad Prism software (version 5.0).

Results: Thrombin induces a rapid calcium mobilization that peaks within 5 seconds and decays back towards baseline fluorescence levels within 2 minutes (Supplementary Fig. S1A, available in the online version). Preliminary studies showed that enoxaparin alone (Supplementary Fig. S1A, available in the online version). Increasing concentration of enoxaparin in the presence of ATIII progressively inhibited thrombin-induced platelet calcium mobilization, with enoxaparin 1 to 2 U/mL almost abolishing the increase in fluorescence (Supplementary Fig. S1B, available in the online version).

Interpretation: Enoxaparin effectively inhibits thrombin-induced platelet activation at therapeutic concentrations in the presence of a physiological concentration of ATIII.

Inclusion and Exclusion Criteria for the PENNY PCI Study

Inclusion Criteria
1. Age ≥ 18.
2. Confirmation of the diagnosis of ST-elevation myocardial infarction (STEMI) on the basis of a classical history, electrocardiographic (ECG) changes and angiographic findings.
3. Pre-treatment with either ticagrelor or prasugrel.
4. Intention to proceed with primary percutaneous coronary intervention (PPCI).
5. Feasibility to obtain informed verbal consent pre-PPCI.

Exclusion Criteria
1. Active bleeding that cannot be controlled by local measures.
2. Pregnant patients.
3. Patients with end-stage renal failure requiring renal replacement therapy.
4. Known thrombocytopenia (platelet count < 10,000/μL).
5. Known history of intra-cranial haemorrhage.
6. Known current treatment with oral anti-coagulants.
7. Known history of major surgery or trauma or history of GI/GU haemorrhage within the last month.
8. Known intra-cranial malignancy or aneurysm.
9. Known allergy to enoxaparin.
10. Known hypersensitivity to benzyl alcohol.
11. Patients with acute bacterial endocarditis.
12. Active gastric or duodenal ulceration.
13. Inability to easily understand verbal information given in English for any reason.
14. Inability to give informed consent due to either temporary or permanent mental incapacity.
15. Current participation, or participation within the last month, in an interventional clinical trial.
Supplementary Fig. S1 Inhibition of thrombin 0.5 U/mL-induced calcium mobilization by enoxaparin (0–2 U/mL). Platelets were labelled with fluo-4 AM and the increase in median fluorescence induced by 0.5 U/mL thrombin was measured by flow cytometry over 2 minutes assessing (A) the response in the absence or presence of 0.125 mg/mL anti-thrombin III (AT3) and enoxaparin 0, 0.125, 0.5 and 2 U/mL and (B) area under the curve (AUC) with enoxaparin 0 to 2 U/mL. Data are mean ± standard error of the mean (SEM), n = 6. Difference in the AUC was determined using analysis of variance (ANOVA). """" p < 0.0001.

Supplementary Fig. S2 The effects of enoxaparin on thromboelastometry. Scatter plots of all thromboelastometry measurements (n = 11). T1: pre-anti-coagulation; T2: end of primary angioplasty; T3: 2 to 3 hours into infusion; T4: end of infusion. """" denotes p < 0.0001, calculated by analysis of variance (ANOVA).