Heparin derivatives in acute coronary syndromes
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Citation for published version (APA):
The impact on coagulation of an intravenous loading dose in addition to a subcutaneous regimen of low-molecular weight heparin in the initial treatment of acute coronary syndromes


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Submitted
Summary

**Objective** - We quantified the impact on coagulation of two different low-molecular weight heparin regimens currently used in the treatment of acute coronary syndromes (ACS).

**Background** - Although low-molecular weight heparin (LMWH) is currently the standard of treatment in ACS, it is unclear if an initial intravenous (IV) loading dose should be added to a subcutaneous (SQ) regimen. We performed a randomized comparison of IV plus SQ versus SQ alone regimen and quantified the impact on the coagulation system in ACS patients.

**Methods** - A total of 25 patients admitted with ACS were randomised to IV plus SQ enoxaparin (n=14) or SQ alone enoxaparin treatment (n=11). Several coagulation markers were determined at nine timepoints during the first 24 hours after treatment start.

**Results** - IV plus SQ immediately resulted in therapeutic anti-Xa levels which remained significantly higher up to 6 hour post-administration compared to SQ alone, without achieving excessively high levels. This was associated with a rapid decrease of plasma levels prothrombin fragments F$_{1+2}$ as soon as 5 minutes after IV injection (33% lower; $p=0.007$), and these levels remained lower up to 2 hours after treatment start compared to SQ alone. The ex-vivo thrombin generation time reached maximum levels at 5 minutes post-injection in the IV plus SQ group and remained significantly prolonged up to 6 hours post-administration compared to SQ alone. TFP I plasma activity was immediately increased by 194% with IV plus SQ while the maximum increase in the SQ alone was 47% at 3 hours.

**Conclusion** - Therapeutic plasma levels of enoxaparin are achieved significantly more rapidly by a combined IV plus SQ regimen compared to SQ alone, without leading to unacceptably high levels of anticoagulation. As the risk of thrombotic complications is greatest in the early hours after admission, the observed differences in thrombin generation suppression may well translate into clinical benefit.

Introduction

Low-molecular weight heparins (LMWH) are now routinely used in the initial treatment of patients admitted with acute coronary syndromes (ACS). However, there is no uniformity regarding the initiation of LMWH,
both in clinical trials and in clinical practice. In several trials\textsuperscript{(1-3)}, treatment was initiated by subcutaneous injection (SQ) on admission. In contrast, other trials\textsuperscript{(4,5)} used a combined regimen, with an intravenous (IV) loading dose followed by SQ administration. An intravenous loading dose may benefit these patients, as the highest event rates are observed early after admission. However, this combined regimen results in higher initial plasma drug levels, potentially increasing the risk of bleeding. No studies have compared these regimens.

Therefore, we quantified the impact on the coagulation system in patients admitted with ACS in a randomized comparison between these two regimens.

Methods

Patient selection and treatment

Patients admitted to the Coronary Care Unit at the Academic Medical Center in Amsterdam, The Netherlands, with a diagnosis of unstable angina or non-Q-wave myocardial infarction were eligible. Not included were patients using anticoagulants including heparin or warfarin, or antiplatelet agents other than aspirin (i.e. clopidogrel, NSAIDS).

After informed consent, patients were randomized to 40 mg IV enoxaparin combined with SQ enoxaparin or SQ enoxaparin alone. In the combined group, subcutaneous enoxaparin was administered within 2 minutes after the IV dose.

Based on a standard dose of 1 mg/kg, the following SQ dose of enoxaparin was administered twice daily: 60 mg if the bodyweight was less than 70 kg, 80 mg if between 70 - 90 kg, and 100 mg if above 90 kg. Aspirin was dosed at 100 mg once daily.

Blood sampling

Nine blood samples were obtained from a dedicated IV catheter during the first day of admission: before administration of enoxaparin, at 5 minutes, and 1, 2, 3, 4, 6, 8, and 24 hours after enoxaparin start. The first 2 ml blood was discarded, and 9 ml blood was collected in citrated Vacutainer tubes (4.5 ml. cit. Na - 0.105 M Silic. 1-10). Blood was centrifuged at 2200 g for 20 minutes at 6 °C. Plasma was separated and filled out in cryocups and frozen at - 80 °C until analysis was performed. These procedures were completed within one hour after blood sampling.
Assays

Anticoagulation levels were determined by an anti-factor Xa determination with a chromogenic assay (Chromogenix) calibrated against enoxaparin.

To measure in-vivo thrombin generation, prothrombin fragment 1 + 2 (F1+2) plasma concentrations were determined by sandwich-type ELISA assay (Dade-Behring, Marburg, Germany). Additionally, the thrombin generation time (TGT) was determined, reflecting ex-vivo thrombin formation using calcium and recombinant human tissue factor (Dade, Innovin). Results were measured spectrophotometrically and expressed as $T\frac{1}{2} \max$ (time to reach the midpoint of clear to maximal turbid density).

Tissue factor pathway inhibitor activity (TFPI), a natural anticoagulant, was quantified by a sandwich-type ELISA from Behring (Marburg, Germany).

Statistical analysis

Differences between the groups were identified using ANOVA analysis. An area under the curve (AUC) between time-points 0 hours and 8 hours was calculated, and compared using an independent sample t-test. Differences between time points within a group were compared using a paired samples t-test.

Results

Baseline characteristics

Of the 25 patients randomized, 14 received IV plus SQ enoxaparin (IV plus SQ group) and 11 SQ enoxaparin alone (SQ group). Baseline characteristics of the two groups were comparable (Table 1).

During the first 24 hours of treatment, no bleeding complications occurred. Six patients in the IV plus SQ group and two patients in the SQ group had recurrent anginal symptoms, of which only 3 patients had accompanying ischemic ECG changes (1 IV plus SQ and 2 SQ patients). In both groups 1 patient underwent coronary angiography, and 1 revascularization was performed (IV plus SQ group).
Table 1 - Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>s.c. alone group</th>
<th>i.v. group</th>
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<tbody>
<tr>
<td></td>
<td>( n = 11 )</td>
<td>( n = 14 )</td>
</tr>
<tr>
<td>Female, n (% )</td>
<td>4 (36)</td>
<td>5 (36)</td>
</tr>
<tr>
<td>Age, years ± s.d.</td>
<td>63±13</td>
<td>63±10</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>28.4</td>
<td>26.5</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>2 (18)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>0 (0)</td>
<td>4 (29)</td>
</tr>
<tr>
<td>Hyperlipidaemia, n (%)</td>
<td>4 (36)</td>
<td>6 (43)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>6 (55)</td>
<td>6 (43)</td>
</tr>
<tr>
<td>History of coronary disease, n (%)</td>
<td></td>
<td></td>
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<tr>
<td>Stable Angina</td>
<td>6 (55)</td>
<td>4 (29)</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>2 (18)</td>
<td>4 (29)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>3 (27)</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Angioplasty</td>
<td>2 (18)</td>
<td>3 (21)</td>
</tr>
<tr>
<td>CABG</td>
<td>2 (18)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Aspirin use on admission, n (%)</td>
<td>9 (82)</td>
<td>7 (50)</td>
</tr>
<tr>
<td>ST elevation and/or depression on admission, n (%)</td>
<td>4 (36)</td>
<td>7 (50)</td>
</tr>
</tbody>
</table>

Anticoagulation levels

Within 5 minutes after IV injection of enoxaparin, mean anti-Xa levels rose from 0 U/ml to 1.25 U/ml (Figure 1). During the following hours, levels gradually decreased to 0.63 U/ml after 8 hours. In contrast, 5 minutes after SQ alone administration, anti-Xa levels were still undetectable, increasing to 0.30 U/ml after 1 hour, and remained significantly lower up to 6 hours compared to the IV plus SQ group. The area under the curve between time points 0 and 8 hours was significantly higher in the IV plus SQ group compared to the SQ alone group (p<0.0005). Anti-Xa levels at steady state (24 hours after baseline) were comparable between both groups (±0.90 U/ml).
In-vitro thrombin generation

Higher initial anti-Xa levels were reflected in the thrombin generation time (Figure 2). A slow increase in TGT was observed after SQ alone injection, rising from 170 seconds at baseline to a maximum of 738 seconds at 6 hours. IV loading resulted in an immediate increase of TGT to ± 2000 seconds 5 minutes after injection, which remained significantly higher during the first 6 hours compared to SQ alone group. This delay in thrombin generation was reflected in a significant higher AUC in the IV plus SQ group compared to the SQ alone group (p<0.0005).

In-vivo thrombin generation

IV bolus injection resulted in a rapid decrease of F\textsubscript{1+2}, with significantly lower levels compared to baseline levels as soon as at 5 minutes post-injection (0.76 nmol/L; p<0.0005), which further decreased in the first 24 hours to 0.58 nmol/L (Figure 3). In the SQ alone group, 2 hours after administration, F\textsubscript{1+2} levels were significantly lower than baseline (p=0.01) and reached the lowest level at 24 hours of 0.71 nmol/L.
Figure 2 - Thrombin generation time (TGT) during the first 24 hours of treatment with subcutaneously alone (black circles) or intravenous plus subcutaneous (white squares) enoxaparin. Data are presented as mean values +/- SEM. Significant differences (p<0.05) using ANOVA between the two groups are marked with an asterisk (*).

Significantly lower F₁+₂ levels between in the IV plus SQ group compared to the SQ alone group were observed at the 5 minutes, 1 hour and 2 hour time points. The AUC between time points 0 and 8 hours was non-significantly reduced by 19% in the IV plus SQ group compared to the SQ alone group (p=0.2).

In-vivo natural anticoagulants

IV enoxaparin resulted in an immediate increase of plasma TFPI, tripling the mean plasma activity from 80% to 294% (Figure 4). Subcutaneous alone administration showed a modest increase of plasma TFPI activity from 79% to a maximum of 126% at 3 hours. During the first 2 hours, TFPI was significantly different between the treatment groups. Six hours after start of treatment, both groups had returned to baseline TFPI levels. A 68% higher AUC in the IV plus SQ group was observed (p=0.1).
Discussion

Our study demonstrates that a combined IV and SQ enoxaparin administration compared to SQ enoxaparin alone as initial treatment of patients with ACS results in a more rapid inhibition of the coagulation system. This is reflected by an immediate decrease of plasma levels of in-vivo thrombin generation marker F\textsubscript{1+2}, and of ex-vivo prolongation of the thrombin generation time. Intravenous initiation of enoxaparin treatment immediately resulted in therapeutic anticoagulation levels, with anti-Xa levels of 1.25 U/ml within 5 minutes after administration. Furthermore, an immediate release of TFPI was observed, tripling TFPI plasma activity. This rapidly induced anticoagulant effect remained significantly greater up to 6 hours after treatment start compared to the SQ alone enoxaparin regimen.

Whether the reduction in thrombin generation during the first hours of ACS treatment results in a reduction of ischemic events cannot be derived from our study. A retrospective comparison of trials using SQ LMWH\textsuperscript{(1-3)} with those using IV plus SQ LMWH\textsuperscript{(4,5)} is not reliable because of the differences in patients and methods. However, patients admitted for ACS
are at highest risk for ischemic events during the first 24 hours, and the CURE study demonstrated that antithrombotic treatment may impact on clinical events as early as four hours after initiation of treatment\(^{(6)}\).

An immediate and significant increase of TFPI activity caused by IV enoxaparin was observed. This rapid release could be of benefit especially in these ACS patients. Coronary plaque rupture exposes subendothelial tissue factor to the circulation\(^{(7,8)}\), which is a strong activator of the coagulation system and local thrombus formation. TFPI binds to the tissue factor-factor VII complex and inhibits the procoagulant properties of TF-fVIIa. TFPI has been shown to reduce TF activity, particularly in human atherosclerotic plaques\(^{(9)}\). We observed no depletion of TFPI activity in the IV plus SQ group as compared to the SQ alone group.

A potential disadvantage of a combined IV and SQ regimen is an increased risk of bleeding complications. We observed maximum mean peak anti-Xa levels in the IV plus SQ group of 1.25 U/ml 5 minutes after treatment start. These levels are comparable with those observed during steady state SQ alone treatment in healthy volunteers (1.2 U/ml)\(^{(10)}\) and ACS patients (1.0 U/ml; range 0.9-1.2 U/ml)\(^{(11)}\). Since the therapeutic range of enoxaparin is 0.5 U/ml - 1.0 U/ml\(^{(10)}\), patients given an IV loading dose are below this
upper limit within 1 hour after administration (Figure 1). It is therefore unlikely that maximum anti-Xa levels after 40 mg IV enoxaparin will induce an unacceptable increased risk of bleeding complications. This is supported by comparison of the data from the TIMI 11B and ESSENCE studies, using a 30 mg IV plus SQ and SQ alone enoxaparin regimen, respectively. The incidence of minor bleeding during the first 24 hours of treatment in the IV plus SQ patients was 1.8% versus 2.1% in the SQ alone patients. Major bleedings occurred in 0.26% versus 0.31% of the patients, respectively. Both this indirect comparison and our data do not suggest that an initial IV loading dose of LMWH is associated with an excessive risk of bleeding that might offset the benefit of achieving therapeutic drug levels early.

In conclusion, therapeutic drug levels were achieved significantly earlier with combined IV plus SQ treatment compared with SQ alone treatment. This resulted in a 2 to 6 hours earlier reduction of in-vivo thrombin generation marker prothrombin fragment 1+2 levels, a delay in thrombin generation time, and early threefold increase of plasma TFPI activity compared to SQ alone enoxaparin. The combined IV plus SQ regimen did not result in unacceptably high anticoagulation levels. This study provides a rationale for a combined IV plus SQ start of LMWH administration in the treatment of ACS patients. This combined regimen may translate into clinical benefit over a SQ alone regimen, although this clinical benefit remains to be determined.
Reference List

Loading dose of LMWH in ACS patients