Rebound Thrombin Generation After Heparin Therapy in Unstable Angina
A Randomized Comparison Between Unfractionated and Low-Molecular-Weight Heparin

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OBJECTIVES This study compared rebound coagulation in patients with acute coronary syndrome patients after discontinuation of unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH).

BACKGROUND Up to a quarter of patients hospitalized for unstable angina experience recurrent ischemia after discontinuation of UFH or LMWH therapy, which may be the result of rebound coagulation activation and subsequent thrombosis. It is unknown whether UFH and LMWH differ in this respect.

METHODS We randomized 71 patients admitted with unstable angina to intravenous UFH or subcutaneous LMWH (dalteparin) and measured plasma markers of coagulation before, during, and after treatment.

RESULTS A complete series of measurements was obtained in 59 patients. Plasma prothrombin fragment 1+2 (F1+2) levels decreased in both groups during treatment. After loss of therapeutic plasma drug levels, F1+2 increased (within 3 h) to a maximum level at 12 to 24 h that was higher than before or during treatment in both groups (p<0.0001). In both groups, F1+2 levels remained higher than pretreatment up to 24 h after discontinuation. Similarly, thrombin-antithrombin (TAT) levels exceeded treatment and pretreatment levels, at a slower rate after dalteparin than after UFH. However, after dalteparin a higher peak value of TAT was observed.

CONCLUSIONS Rebound coagulation activation occurs within hours after discontinuation of both UFH and dalteparin. With both drugs, thrombin generation is significantly greater after treatment than before or during treatment. A longer duration or weaning of treatment, or continuation with another anticoagulant treatment, may reduce rebound coagulation activation and ischemic events. (J Am Coll Cardiol 2002;39:811–7) © 2002 by the American College of Cardiology Foundation
might avoid or reduce this phenomenon. Clinically superior results of LMWH over UFH in patients with ACS could partially be based on such a difference (1–4). To date, a rebound phenomenon has not been reported for LMWH. Therefore, we performed a randomized study to determine whether rebound activation of coagulation occurs after cessation of LMWH treatment, and whether this is different from rebound phenomenon seen with UFH.

METHODS

Patient selection. Consecutive patients admitted to our coronary care unit for non–ST-segment elevation ACS were screened for eligibility. Inclusion criteria were rest angina of at least 5 min in duration and either electrocardiogram (ECG) changes compatible with acute ischemia (ST-segment depression ≥0.1 mV in ≥2 contiguous leads, T-wave inversion ≥0.1 mV, transient ST elevation or new left bundle branch block), creatinine kinase-MB (CK-MB) levels of at least twice the normal limit or a history of documented coronary artery disease. Excluded were patients with known coagulation disorders, using anticoagulant agents or scheduled to be transferred before the end of the study.

Informed consent was obtained from all patients. Randomization was performed with sealed envelopes containing treatment allocation. The study was approved by the Institutional Review Board of the Academic Medical Center in Amsterdam.

Treatment. Unfractionated heparin treatment started with an intravenous (IV) bolus of 5,000 U, followed by continuous infusion to reach a partial thromboplastin time between 60 and 90 s (i.e., 2- to 2.5-fold increase of control value). Low-molecular-weight heparin treatment consisted of twice daily subcutaneous injections 120 IU/kg dalteparin (Fragmin). All patients received aspirin 100 mg daily. By protocol, patients were treated with study medication until clinically stable, for at least 48 h from onset of treatment or, if applicable, at least 48 h after catheterization.

Additional medication was left to the discretion of the treating physician, but included beta-blockers and IV nitroglycerin. Patients underwent coronary angiography only if they developed severe refractory ischemia.

Blood sampling. Blood samples were collected before start of study medication, during steady-state anticoagulation (24 h after start of study medication) and at 0, 3, 6, 12, 24 and 48 h after loss of therapeutic levels of the respective heparins. For patients treated with UFH, cessation of treatment was defined as time of discontinuation of the infusion. For patients treated with dalteparin, cessation of treatment was defined as 12 h after the last subcutaneous injection (Fig. 1).

Blood samples were obtained through an IV infusion system that was distal to other systems and not used for medication. At each sampling, the first 2 ml of blood was discarded and 10 ml was collected in citrated Vacutainer tubes (4.5 ml citrate sodium –0.105 M siliconated 1 to 10). Blood was centrifuged at 3,000 rpm for 20 min at 6°C. Plasma was separated, pooled and filled out in cryocups and

Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>ACS</td>
<td>acute coronary syndromes</td>
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<tr>
<td>APC</td>
<td>activated protein C</td>
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<td>CK-MB</td>
<td>creatinine kinase-MB</td>
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<td>ECG</td>
<td>electrocardiogram</td>
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<td>F1\textsubscript{1+2}</td>
<td>prothrombin fragment 1+2</td>
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<td>IV</td>
<td>intravenous</td>
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<td>LMWH</td>
<td>low-molecular-weight heparin</td>
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<td>PAP</td>
<td>plasmin-antiplasmin complexes</td>
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<td>TAT</td>
<td>thrombin-antithrombin complexes</td>
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<tr>
<td>TFPI</td>
<td>tissue factor pathway inhibitor</td>
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<td>UFH</td>
<td>unfractionated heparin</td>
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Figure 1. Schematic anticoagulation levels of unfractionated heparin (UFH) and low-molecular-weight heparin and definition of discontinuation time point for UFH and dalteparin. (a) last dose of dalteparin; (b) cessation of UFH, discontinuation time point; (c) discontinuation time point for dalteparin.
frozen at −80°C until analysis was performed. These procedures were completed within 1 h after blood sampling. Assays. Quantification of prothrombin fragment 1+2 (F1+2), thrombin-antithrombin complexes (TAT) and plasmin-antiplasmin complexes (PAP) was performed by sandwich-type enzyme-linked immunosorbent assays (Dade-Behring, Marburg, Germany). Upper normal values of these markers were F1+2 1.6 nmol/l, TAT 4.6 μg/l and PAP 700 μg/l.

Levels of heparin activity were determined by a microtitre plate chromogenic anti-factor Xa activity assay (Chromogenix) and calibrated against UFH or dalteparin.

Clinical outcome. Recurrent ischemic events during hospitalization were classified as angina with ischemic ECG changes, angina leading to angiography or revascularization, reintervention with CK-MB rise of at least twice the normal limit and death.

Statistical analysis. To determine differences between time points within a treatment group, a nonparametric related samples test (Wilcoxon) was performed for each of the eight time points relative to the preceding time point. For comparisons between the UFH and dalteparin groups, a nonparametric independent samples test (Mann-Whitney U test) was calculated. All statistical analyses were performed with SPSS 10.0.7 (Chicago, Illinois). To examine the absolute change of a parameter after treatment cessation, stop values were subtracted from the following values and were analyzed using a nonparametric independent samples test (Mann-Whitney U test).

No formal repeated measurement analyses could be performed because the data were not normally distributed.

Patients were excluded from the analysis when more than two blood samples were missing, unless this was due to the occurrence of a recurrent ischemic event resulting in therapeutic intervention (angioplasty, restart of anticoagulation).

Highly elevated levels of F1+2, TAT and PAP were considered as sampling errors with ex-vivo coagulation activation and excluded from the analysis. Cut-off values for F1+2 were 8 nmol/l, TAT 50 μg/l and PAP 1,000 μg/l. This resulted in the exclusion of 1%, 6% and 6% of the total F1+2, TAT and PAP samples, respectively.

RESULTS

Of 71 study patients, 38 were randomized to UFH and 33 to dalteparin. Twelve patients were not included in the analysis because they had more than two missing blood samples. The reasons for incomplete blood sampling were transfer to other hospitals (n = 4), continuation of open-label UFH or LMWH due to angioplasty or planned coronary artery bypass graft (n = 6), at patient’s request (n = 1) or at doctor’s request (n = 1).

Table 1 summarizes baseline characteristics of the 59 included patients. No significant differences between the UFH and dalteparin groups were observed. Median duration of treatment from first through the last dose was 48 h for UFH patients and 55 h for dalteparin patients. Patients treated with UFH had median prothrombin times of 66 s at 24 h after start and 68 s immediately before discontinuation of therapy. While on anticoagulant treatment six UFH and seven dalteparin patients underwent coronary angioplasty, after which treatment was continued for a median of 44 h.

Thrombin generation. On admission, F1+2 plasma levels were comparable in both groups (Fig. 2). During the first 24 h of treatment, both UFH and dalteparin significantly decreased F1+2 levels (p < 0.02), although dalteparin resulted in a greater decrease compared to UFH (p = 0.002). At the end of the treatment period, F1+2 levels in the dalteparin group were not significantly different from those in the UFH (see time point “stop”). No significant changes of F1+2 were observed in the UFH group during the remaining treatment period.

In the UFH group, treatment cessation resulted in a rapid increase of F1+2, from 0.74 nmol/l to 1.25 nmol/l 3 h after stopping (p < 0.001). This increase persisted through 6 and 12 h after stop and reached a maximum level of 1.48 nmol/L at 12 h. Dalteparin cessation also resulted in an increase of thrombin generation with similar time to peak levels at 12 h (1.21 nmol/l) after cessation. Compared to UFH, F1+2 levels were lower at 3 and 6 h after of dalteparin (p = 0.002 and p = 0.02, respectively) and remained lower although the 12, 24 and 48 h samples were not significantly different. At 48 h after treatment discontinuation, F1+2 levels in both groups were at a similar level. When we corrected for F1+2 measured at the stop time point, no differences in F1+2 generation between UFH and dalteparin were observed. Both groups revealed significant increases of F1+2 levels with a maximum increase of 0.52 nmol/l at 6 h in the UFH group and of 0.61 nmol/l at 12 h in the dalteparin group (both p < 0.0001 compared to stop values).

Compared to pretreatment levels, both groups showed increased F1+2 levels up to 24 h after treatment discontinuation (p < 0.03).

Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>UFH n (%)</th>
<th>Dalteparin n (%)</th>
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<tbody>
<tr>
<td>Female/male</td>
<td>9/20</td>
<td>14/16</td>
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<tr>
<td>Age (yrs)</td>
<td>63</td>
<td>61</td>
</tr>
<tr>
<td>Weight (mean in kg)</td>
<td>83</td>
<td>78</td>
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<tr>
<td>Diabetes</td>
<td>7 (24)</td>
<td>7 (23)</td>
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<td>Smoking</td>
<td>13 (45)</td>
<td>11 (37)</td>
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<tr>
<td>Previous hyperlipidemia</td>
<td>6 (21)</td>
<td>13 (43)</td>
</tr>
<tr>
<td>Previous hypertension</td>
<td>11 (38)</td>
<td>13 (43)</td>
</tr>
<tr>
<td>Previous stable angina</td>
<td>10 (34)</td>
<td>16 (53)</td>
</tr>
<tr>
<td>Previous unstable angina</td>
<td>9 (31)</td>
<td>14 (47)</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>7 (24)</td>
<td>13 (43)</td>
</tr>
<tr>
<td>Previous angioplasty</td>
<td>6 (21)</td>
<td>12 (40)</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>2 (7)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Previous aspirin use</td>
<td>14 (50)</td>
<td>21 (70)</td>
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<tr>
<td>Angioplasty during heparin treatment</td>
<td>6 (21)</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Median administration of anticoagulant treatment (h)</td>
<td>48</td>
<td>55</td>
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CABG = coronary artery bypass graft; UFH = unfractionated heparin.
Thrombin-antithrombin complex formation. Figure 3 presents the median plasma levels of TAT in both groups. Pretreatment levels were comparable in both groups (7.3 µg/l in UFH and 5.4 µg/l in dalteparin). Dalteparin treatment did not result in changes of TAT levels 24 h after treatment start, whereas UFH treatment resulted in a level increase from 7.3 µg/l to 13.8 µg/l (p = 0.121), which was significantly different between both groups (p = 0.042). Continuation of UFH treatment restored plasma TAT levels similar to those in the dalteparin group at 48 h after treatment start (7.2 µg/l and 6.1 µg/l, respectively, p = 0.3).

When treatment with UFH was stopped, a rapid increase of TAT levels was observed at 3 h, reaching a maximum at 6 h after discontinuation (20.6 µg/l). At 12, 24 and 48 h, TAT levels decreased to 13.5 µg/l, 12.2 µg/l and 4.8 µg/l, respectively. In the dalteparin group, a slight increase was observed 3 h after treatment stop, with a steep increase at 6 and 12 h to TAT levels of 15.2 µg/l and 31.4 µg/l, respectively. At 24 h, TAT levels were similar in both groups, and the 48 h levels were comparable with pretreatment levels. Differences between UFH and dalteparin were observed at 3 and 12 h after treatment stop (p = 0.01 and p = 0.004, respectively). When we corrected for TAT levels measured at the stop time point, only dalteparin differed from UFH at the 12-h post-treatment time point (p = 0.01).

Fibrinolysis. Plasmin-antiplasmin levels, an indicator of fibrinolytic system activity, are presented in Figure 4. Both UFH and dalteparin effectively suppressed the generation of PAP complexes, indicating a decrease in fibrinolysis during treatment (p < 0.001), which persisted until treatment discontinuation. Cessation resulted in both groups to marked and persistent increases of PAP levels that were still
r спустя 48 ч и были достоверно выше, чем до лечения (p < 0.02).

**Anti-Xa levels.** Anti-Xa уровни, индикатор активности гепарина, измерялись в течение всего времени и медиановые значения представлены на рисунке 5. Величина Anti-Xa уровней была достоверно различна между стационарным лечением с далтепарином (0.74 U/ml) и UFH (0.30 U/ml) (p = 0.004). На завершение лечения, Anti-Xa уровни были сопоставимы, согласно предположению, что через 12 ч после последнего подкожно введения далтепарина, терапевтический уровень антикоагуляции все еще присутствует. Более плавное снижение Anti-Xa уровней было отмечено в группе далтепарина, с Anti-Xa уровнями 0.23 U/ml и 0.12 U/ml на 3 и 6 ч соответственно (p < 0.0001 и p = 0.26 по сравнению с UFH, соответственно). Anti-Xa было почти недетектабельным в группе UFH через 3 ч после завершения инфузии (0.01 U/ml).

**Clinical recurrent ischemic events.** Семь пациентов испытывали рецидивирующие инфаркты миокарда с изменениями ЭКГ за 48 ч после завершения лечения (три пациента UFH и четыре пациента далтепарин). Все были без событий в течение периода лечения. В трех случаях, антикоагуляционная терапия была снова введена, и одна срочная реваскуляризация была выполнена. Ни один из пациентов не умер или не перенес инфаркт миокарда в течение первых 48 ч после лечения, хотя один UFH пациент погиб позднее в госпитализации. Четыре из семи пациентов показали F1+2 увеличение свыше верхней границы нормы после прекращения (1.6 нмоль/л, медианное пиковое значение 6.0 нмоль/л) одновременно или предшествуя ему до 6 ч, в то время как 28 пациентов без рецидивирующих инфарктов миокарда также показали увеличение F1+2 (среднеквадратичное значение 2.2 нмоль/л). Уровни TAT после прекращения были обычными в обоих группах (в среднем, хотя бы в одном из наблюдений).
one patient) and levels were similar in patients with and without recurrent angina (median level of 33.4 μg/l and 31.7 μg/l, respectively).

DISCUSSION

This study demonstrates that rebound activation of the coagulation system occurs after discontinuation of both UFH and dalteparin treatment, and it is the first to compare the two. We found that the time course and the magnitude of this rebound phenomenon was not different for UFH and dalteparin. The peak level of F$_{1+2}$ observed after discontinuation of dalteparin compared to UFH was similar after correction for stop F$_{1+2}$ values, indicating similar absolute increases of F$_{1+2}$. After both treatments, a sharp increase in thrombin formation and TAT complexes was observed. This was also reflected in plasma PAP complexes, which increased up to 48 h after termination of heparin administration. Remarkably, levels of F$_{1+2}$ and PAP remained elevated at least 24 h after treatment was discontinued, and these levels were significantly higher than levels before treatment was started, suggesting a heparin-induced elevation of these markers.

Differences between UFH and dalteparin were evident during treatment, when dalteparin was more effective in decreasing F$_{1+2}$ levels. Thrombin-antithrombin levels were unaffected by dalteparin treatment, but in the UFH group a significant increase was observed. In both groups PAP levels decreased. The more effective suppression of F$_{1+2}$ by dalteparin is consistent with the higher level of anti-Xa activity (Fig. 5). Stable UFH levels are usually not reached within 24 h, whereas dalteparin results in predictable levels immediately after treatment is started. This difference is likely reflected in the amount of F$_{1+2}$ formed. Although one would expect TAT levels to show a similar pattern, no change was observed with dalteparin treatment, whereas UFH resulted in a rise of these complexes (Fig. 3). Although subtherapeutic levels of anticoagulation will fail to decrease TAT levels, this cannot explain the increase of the plasma levels as seen in the UFH group. Therefore, LMWH administration may affect TAT complex formation in a different way.

Our study was too small to detect clinical differences between the treatment groups. There was no clear relation between clinical recurrent angina and increases of F$_{1+2}$ and TAT. However, peak levels of F$_{1+2}$ were higher in patients with recurrent angina (6.0 nmol/l vs. 2.2 nmol/l), suggesting that the magnitude of thrombin generation may contribute to the recurrence of clinical ischemia.

Previous studies. Rebound thrombin generation after discontinuation of UFH has been investigated previously (13–15). In a study by Granger et al. (13), UFH cessation resulted in increases of both F$_{1+2}$ and fibrinopeptide A at 3 to 6 h. Normalization of all markers to pretreatment levels was observed, whereas we observed that only TAT levels decreased to baseline values at 48 h after cessation. Becker et al. (14) found increased thrombin generation as soon as 1 h after UFH cessation and further increasing at 24 h. Other studies have shown that coagulation parameters may remain elevated during the weeks or even months after the onset (and treatment) of ACS (16–18).

Thrombin generation observed after discontinuation of heparin is not likely explained by returning to the natural course of the disease. In a Fragmin and Fast Revascularisation during Instability in Coronary artery disease (FRISC) substudy, placebo-treated patients showed no significant changes in plasma F$_{1+2}$ levels in the first five days after admission (16).

Potential mechanisms. A state of hypercoagulability after heparin treatment may be induced by tissue factor pathway inhibitor (TFPI) depletion (19). This lipoprotein, bound to endothelial cells, is a natural anticoagulant inhibiting both factor Xa as well as the tissue factor/VIIa/fXa complex. Infusion of heparin releases TFPI from endothelial cells, and continued administration of heparin may result in depletion of this anticoagulant. We did not perform endothelium-bound TFPI measurements because UFH boluses are required for quantification of this protein. Second, both UFH and LMWH inhibit the generation of activated protein C (APC), resulting in low levels of this natural anticoagulant during treatment (20). Cessation of anticoagulation at low APC levels may promote rebound coagulation.

It is conceivable that angioplasty would stabilize the culprit lesion and thus prevent rebound phenomena. However, in our study, among 8 of 13 patients who underwent angioplasty during treatment, discontinuation of therapy resulted in an increase of F$_{1+2}$ above 1.6 nmol/l, suggesting that the intervention may not result in a different rebound pattern.

Clinical implications. Rebound thrombotic phenomena have important clinical implications (7–11). Several clinical studies have investigated the effect of prolonged treatment with either LWMH (2,3,4,21) or oral anticoagulant treatment after ACS (22–24). In the FRISC II study, a subgroup of patients with ACS who continued dalteparin treatment up to 90 days after hospital discharge did not benefit from this regimen compared to short-term dalteparin (21). However, when high risk patients were selected on the basis of troponin T-levels and ST-segment shifts on admission, a significant reduction of death alone or death and myocardial infarction at 90 days was obtained (25). It is unclear if our findings with dalteparin can be extrapolated to other LMWHs. In clinical studies, differences in outcome have suggested that LMWHs are not equally effective (1–4,21). However, variations in the designs of the studies may explain these differences, and a direct comparison is lacking. There have been no observations on rebound coagulation after discontinuation of other LMWHs.

A recent publication focused on long-term oral anticoagulation in patients discharged after hospitalization for ACS (23). In patients compliant with the medication, oral
Anticoagulants resulted in a reduction of the combined end point cardiac death, myocardial infarction or stroke (risk ratio 0.68, 95% confidence interval 0.48 to 0.95). Cessation of oral anticoagulation did not result in a rebound of ischemic events during one-month follow up.

Because aspirin has been shown to reduce rebound effects (8), additional inhibitors of platelet function may further reduce rebound thrombosis. However, of patients in our study who received a thienopyridine because of stent implantation, two showed increases of F₁₋₂ above normal limits after discontinuation of heparin treatment.

**Summary.** Treatment with both UFH and dalteparin is followed by reactivation of coagulation to levels higher than before or during treatment, and persisting at least 48 h after treatment discontinuation. In dalteparin-treated patients, reactivation peak levels were not different from those seen after discontinuation of UFH. Rebound thrombin generation in patients with unstable angina or myocardial infarction is likely the cause of clinical recurrent ischemia seen shortly after treatment cessation.

**References**


