CHAPTER 7

Low molecular weight heparin is equally effective as unfractionated heparin in reducing coagulation activity and perfusion abnormalities during the early treatment of pulmonary embolism

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SUMMARY

BACKGROUND: Little is known about differences between UFH and LMWH on coagulation activity during treatment for pulmonary embolism. The objective of this study was to compare UFH and LMWH in the early treatment of pulmonary embolism in terms of control of coagulation markers and perfusion abnormalities.

METHODS: 37 patients with acute pulmonary embolism were randomised to receive intravenous UFH or subcutaneous dalteparin, both together with acenocoumarol. Daily samples were obtained for measurement of thrombin generation (fragments 1+2, TAT-complexes and fibrin monomers) and fibrinolysis (D-dimer concentrations and clot lysis times). Ventilation-perfusion scintographies were performed from which the percentage-of-vascular-obstruction scores (PVOs) were calculated at day 0 (PVOsD0) and day 5 (PVOsD5).

RESULTS: The INR was therapeutic in both groups at day 3. At day 3, anti-Xa levels were 0.21 IU/ml in the UFH group (n=19) and 0.29 IU/ml in the LMWH group (n=18) (p=0.1). F1+2 and TAT-complexes rapidly normalised without differences between the groups (p=0.5 and p=0.4 resp.). Fibrin monomer levels did not decrease, and showed an increase in the UFH group from day 3 (p<0.05 for differences between the groups). D-dimer levels decreased over time, without differences between the groups (p=0.6). Clot lysis times were shorter in the UFH group (p<0.05). The PVOsD0 and PVOsD5 were not different (p=0.5 and p=0.8 resp), but the decrease of the PVOs over time was higher in the LMWH group (p=0.04).

CONCLUSION: LMWH is at least equally effective as UFH in reducing coagulation activity and perfusion abnormalities in the early treatment of pulmonary embolism.
INTRODUCTION

Intravenous administration of unfractionated heparin (UFH) has proven its efficacy in the early treatment of venous thromboembolism (1-3). The purpose of using heparin in the treatment of thrombosis is to inhibit thrombin generation. The use of UFH, however, has several inconveniences. Intravenous administration of UFH requires hospitalisation and frequent monitoring of the activated partial thromboplastin time (aPTT) for optimal dose-adjustment. Low molecular weight heparin (LMWH) has the advantage of a subcutaneous administration route, more stable plasma heparin concentrations, the absence of the need for monitoring and a lower incidence of heparin-induced thrombocytopenia (4). Treatment of deep venous thrombosis of the leg with LMWH is considered to be equally safe as UFH when looking at clinical outcomes (5-20). LMWH also appears to be safe in the treatment of pulmonary embolism from a clinical point of view (10;16;21-24). Less agreement exists about the effect of LMWH versus UFH in reducing coagulation activity in venous thrombosis. Some studies report a more rapid decrease in coagulation activity by UFH as compared to LMWH (25;26), whereas others find the opposite (27;28) or no differences (29-31). These studies mainly concern patients with deep venous thrombosis or healthy donors. The effect of LMWH versus UFH on early changes in haemostatic markers in patients with pulmonary embolism has not yet been studied. As many clinicians still use UFH in the treatment of pulmonary embolism, the finding of equal or more effectiveness of LMWH on coagulation markers and perfusion abnormalities might further support the use of LMWH in the initial treatment of pulmonary embolism.

The purpose of this study is to compare UFH and LMWH in their capacity to inhibit thrombin generation in the early treatment of pulmonary embolism by measuring prothrombin fragments 1+2 (F1+2), thrombin-antithrombin complexes (TAT) and fibrin monomers. Secondly, we compare UFH and LMWH in their effects on fibrinolysis by measuring D-dimer concentrations and clot lysis times. Finally, we compare UFH and LMWH in terms of changes in perfusion abnormalities during the early days of treatment for pulmonary embolism.
PATIENTS AND METHODS

Study design
This was a randomised, non-blinded study where continuous infusion of dose adjusted unfractionated heparin was compared to a once daily subcutaneous injection of dalteparin in terms of changes in coagulation markers and changes in perfusion abnormalities. Informed consent was obtained from all participating patients and the medical ethical committee of our institute approved the study protocol. The primary endpoint of this study was the time course of different coagulation and fibrinolytic markers in the two heparin regimens. The secondary endpoint was the evaluation of changes in perfusion abnormalities during the early treatment with the two heparins.

Patients and study protocol
Patients with proven symptomatic pulmonary embolism (as described later) were eligible for this study. Exclusion criteria were active malignancy (defined as currently receiving any treatment for cancer or documented recurrent or metastatic disease), recent surgery or large trauma in the past month, the use of any form of anticoagulant in the past month, pregnancy, a history of heparin-induced thrombocytopenia and the presence of a contraindication for anticoagulation. Patients were randomised to receive UFH or LMWH (dalteparin). UFH was given within 1 hour after diagnosis as an intravenous bolus of 5000 IU, followed by continuous infusion, starting with 25000 IU/d. Dosage adjustment was made by monitoring the ratio of the activated partial thromboplastin time (aPTT). The first aPTT ratio was measured 6 hours after the start of UFH and was followed by monitoring twice a day with a target range of 2.0-3.5. In patients who were randomised for LMWH, dalteparin was given through a subcutaneous injection within 1 hour after diagnosis and after that once a day at 17h00. Dosages of dalteparin were body weight-adjusted: < 55 kg: 10.000 IU; 55-65 kg: 12.500 IU; 65-85 kg: 15.000 IU; > 85 kg: 18.000 IU. Within 24 hours of the start of the study, oral coumarin derivates were started (acenocoumarol). Treatment with UFH or dalteparin was stopped when the International Normalized Ratio (INR) was > 2.0 on two consecutive days with a minimum duration of five days.
Laboratory assessments
Before the start of any anticoagulant therapy, and following daily between 08h00 and 09h00 the next consecutive 5 days, blood was drawn. The day of presentation and before treatment was started, was considered to be day 0. The INR was measured daily using the Hepato Quick® (Diagnostica Stago, Asnière, France) and the aPTT using the Cephotest™ (Axis-Shield PoC AS, Oslo, Norway). Extra samples were stored and frozen at –70°C until further assessment. Anti-Xa levels were measured at day 3 using the Staclot® Heparin (Diagnostica Stago, Asnière, France).

Thrombin generation was estimated by measurement of prothrombin fragments 1+2 (F1+2) using the ELISA Enzygnost® F1+2 (Dade Behring, Schwalbach, Germany) with normal values 0.4-1.1 nmol/l, thrombin-antithrombin complexes (TAT) using the ELISA Enzygnost® TAT micro (Dade Behring, Schwalbach, Germany) with normal values 1.0-4.1 µg/l and fibrin monomers using Berichrom® FM (Dade Behring, Schwalbach, Germany) with normal values <3.4-14.5 mg/l.

Effects on fibrinolysis were estimated by measuring D-dimer concentration using the ELISA Asserachrom® (Diagnostica Stago, Asnière, France) with normal values < 500 µg/ml Fibrin Equivalent Units (FEU) and plasma clot lysis times using previously described methods (32;33), where lysis of a tissue-factor induced clot by exogenous t-PA was studied by monitoring changes in turbidity during clot formation and subsequent lysis. The contribution of thrombin activatable fibrinolysis inhibitor (TAFI) to the clot lysis time was assessed after addition of 25 µg/ml carboxypeptase inhibitor (CPI, Calbiochem, La Jolla, CA) to the plasma, which is a specific inhibitor of activated TAFI (34).

Laboratory assessments were done by skilled personnel who were unaware of the treatment regimens.

Diagnosis of pulmonary embolism
In all patients, a ventilation-perfusion scintigraphy was performed at presentation (day 0). Lung scans were performed in 4 standard views: anterior, posterior and right and left posterior oblique. Additional right and left lateral views were performed at indication. For interpretation of the scans, Hull’s criteria were used (35), as well as a lung segment reference chart (36). A normal scintigraphy ruled out pulmonary embolism, where a high probability scan confirmed the diagnosis. An intermediate probability scintigraphy was followed by pulmonary angiography. During this angiography, a stan-
standard intravenous dose of 5,000 IU UFH was given. The ventilation-perfusion scintigraphy was repeated at day 5. These results were compared with the results obtained at day 0 by two nuclear specialists who were unaware of the treatment regimens. The percentage-of-vascular-obstruction score (PVOs), as described previously (37;38), was calculated at day 0 (PVOsD0) and at day 5 (PVOsD5).

**Statistics**
For comparison of baseline characteristics between the two groups, the Fisher’s exact test and the Mann-Whitney U test were used with two-tailed p-values. We used the repeated measurement MANOVA for comparison of groups over time with the SAS software (SAS version 8.2, Cary, NC, USA). The Mann-Whitney U test with two-tailed p-values was used to compare daily values between the groups using SPSS software (SPSS version 10.0.5, Chicago, USA). Bivariate correlation between laboratory values and PVOs was calculated using SPSS software.
RESULTS

Forty patients with proven pulmonary embolism were included in this study. In three patients, more than one sample was missing; these 3 patients were excluded from further analysis. Of the remaining 37 patients, 19 patients received UFH and 18 patients received LMWH. For baseline characteristics, see Table 1. The two groups were comparable considering gender, the PVOs and the number of angiographies performed. Patients receiving dalteparin were of older age. Except for F1+2 levels, which were higher in the LMWH group, the coagulation and fibrinolytic markers were comparable. The INR reached therapeutic values in both groups at day 3: the median in the LMWH group was 2.3 and in the UFH group 2.1 (p=0.4), the means were 2.4 ± 0.8 and 2.1 ± 0.8. The mean aPTT ratio in the UFH group after 24 hours was 2.0 ± 0.7, where 8/19 (42%) had values > 2.0. At day 3, 15/19 (79%) had values > 2.0, at day 4 17/19 (89%) and at day 5 all patients had an aPTT ratio > 2.0. Anti-Xa levels at day 3 were 0.29 ± 0.15 IU/ml in the LMWH group and 0.21 ± 0.1 IU/ml in the UFH group (p=0.1). No bleeding complications were observed in the total study population.

Table 1. Baseline characteristics in patients with pulmonary embolism initially treated with intravenous unfractionated heparin or subcutaneous dalteparin. Values are reported as median, unless indicated. (F1+2 = fragments 1+2, TAT = thrombin-antithrombin, FEU= Fibrin Equivalent Units, PVOs = percentage-of-vascular-obstruction score)

<table>
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<th>Dalteparin N = 18</th>
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<td>PVOs (%)</td>
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Evaluation of thrombin generation

The levels of F1+2 decreased over time in the total group (p<0.0001) (Figure 1a). Baseline values were higher in the LMWH group as to compared to the UFH group (p=0.03). After treatment, F1+2 levels normalised rapidly in both groups. No differences were seen at day 1 (p=0.3), day 2 (p=0.052), day 3 (p=0.2), day 4 (p=0.4) and day 5 (p=0.2).

A rapid normalisation of TAT complexes was seen in both groups compared to baseline values (p=0.02) (Figure 1b). After day 1, the levels of TAT complexes remained normal. Between the groups, there was no difference over time (p=0.5). Comparison of daily values between the groups showed no significant differences (p=0.9 at day 0, p=0.3 at day 1, p=1.0 at day 2, p=0.4 at day 3, p=0.4 at day 4 and p=0.3 at day 5).

Fibrin monomer levels did not decrease to normal values during treatment in both heparin regimens. They remained stable in the LMWH group, but increased again in the UFH group. Differences were significant at day 3 (p=0.04), day 4 (p=0.04) and day 5 (p=0.003) (Figure 1c).
Figure 1. The effect of low molecular weight heparin (LMWH) and unfractionated heparin (UFH) on thrombin generation in patients with pulmonary embolism. Dotted lines represent upper normal values.

a) Fragment 1+2 levels (F1+2), b) Thrombin-antithrombin (TAT)-complexes, c) Fibrin monomer (FM) levels
Evaluation of fibrinolysis

The course of the D-dimer levels showed a significant decrease in both groups over time (p<0.0001), but there were no differences between the two groups over time (p=0.6) (Figure 2a). Comparing daily values, there were no differences between the groups (p=0.7 at day 0, p=0.7 at day 1, p=0.9 at day 2, p=1.0 at day 3, p=0.8 at day 4 and p=0.6 at day 5). Comparing daily values, there were no differences between the groups (p=0.7 at day 0, p=0.7 at day 1, p=0.9 at day 2, p=1.0 at day 3, p=0.8 at day 4 and p=0.6 at day 5).

Plasma clot lysis times are shown in Figure 2b. There was a significant difference between the groups over time, with longer clot lysis times in the LMWH group (p<0.0001). Lysis times did not differ at day 0 and 1 (p=0.9 and p=0.1 respectively), but from day 2 they were longer in the LMWH group (p=0.003 at day 2, p<0.001 at day 3, p=0.001 at day 4 and p=0.02 at day 5).

After addition of CPI, there was a similar decrease in clot lysis times at day 0 in both groups (p=0.28). Clot lysis times between the groups were not different at day 0 and day 1 (p=0.052 and p=0.06 respectively), but they were higher from day 2 in the LMWH group (p=0.007 at day 2, p=0.001 at day 3, p=0.006 at day 4 and p=0.048 at day 5). There was no additional effect of CPI on clot lysis times after the start of UFH. In the LMWH group, CPI resulted in shorter clot lysis times at day 0 and day 1 (p=0.001 and p=0.04 respectively), but not at day 2, 3, 4 and 5 (p=0.2, p=0.2, p=0.1 and p=0.1 respectively).
Figure 2. The effect of low molecular weight heparin (LMWH) and unfractionated heparin (UFH) on fibrinolysis in patients with pulmonary embolism. Dotted line in figure 2a represents upper normal value. CPI = carboxypeptase inhibitor
a) D-dimer levels, b) Clot lysis time
Perfusion abnormalities
Six patients did not have a second ventilation-perfusion scintigraphy at day 5 (3 in the UFH and 3 in the LMWH group). They were excluded from PVOs analysis. In the remaining 31 patients, the median of the PVOsD0 was 28.6% in the LMWH group and 22.9% in the UFH group (p=0.5) (Figure 3). At day 5, the values were 15.0% and 23.2% respectively (p=0.8). In the LMWH group, there was a reduction in PVOs although not statistically significant (p=0.09). In the UFH group, no reduction was seen (p=0.6). Although values at day 0 and 5 did not differ statistically between the groups, there was a significant difference in the decrease of the PVOs over time in favour of the LMWH group (p=0.04).
There was a correlation between the PVOsD0 and the initial F1+2 levels at presentation (p=0.009) and between the PVOsD0 and the initial D-dimer levels at presentation (p=0.04). No correlation was found between the PVOsD0 and other laboratory variables. There was no correlation between the PVOsD5 and any laboratory marker at day 5.

Figure 3. Percentage-of-vascular-obstruction score (PVOs) at day 0 (PVOsD0) and day 5 (PVOsD5) in patients with pulmonary embolism treated with low molecular weight heparin (LMWH) or unfractionated heparin (UFH)
DISCUSSION

Inhibiting thrombin generation is the main purpose of treatment of venous thrombosis with heparins. The safety of UFH and LMWH in the treatment for venous thrombosis has been demonstrated in a number of trials (5-24). Few data exist on the effectiveness of both heparin regimens in controlling coagulation markers in deep venous thrombosis. A recent large report showed the efficacy of LMWH in reducing thrombin generation at 1 and 3 weeks (39), but for pulmonary embolism no such studies have been conducted.

In this study, we demonstrate that in patients with pulmonary embolism, both UFH and LMWH immediately decrease levels of F1+2 fragments and TAT-complexes to normal values. The decrease by UFH is in agreement with previous reports (40,41) and the similar decrease in both heparin regimens has been found by others (30,31). The DVTENOX trial, however, showed more rapid decreases of TAT-complexes and F1+2 in patients treated with enoxaparin compared to UFH (27). On the other hand, in a smaller study, Stricker et al found the opposite (25). Some authors seek an explanation for the differences between the studies in the degree of anticoagulation and the moment of reaching therapeutic ranges (25;27).

Interestingly, fibrin monomer levels did not decrease in both heparin regimens. In patients treated with UFH, they even showed an increase after 3 days of treatment. This might implicate that UFH inhibits fibrin formation in a lesser extent over time than LMWH. As the increase in fibrin monomers in UFH is seen from day 3, this cannot be explained by non-therapeutic ranges of UFH, as therapeutic ranges were reached after 3 days. However, anti-Xa levels in the LMWH group were higher at day 3; although this difference was not statistically significant, it might be clinically relevant. One study reported higher anti-Xa levels and lower fibrin monomer levels during infrainguinal bypass surgery in patients treated with LMWH compared to UFH (42). Another explanation may be found in fibrin-bound thrombin. After generation of fibrin, activated thrombin can bind to fibrin. This fibrin-bound thrombin retains its procoagulant activity, but is protected from antithrombin-induced inhibition through the formation of ternary thrombin-fibrin-heparin complexes (43-46). Furthermore, fibrin is a potent modulator of heparin activity in vivo by inhibiting heparin-catalyzed thrombin-antithrombin complex formation through formation of these ternary complexes (47). One could postulate that the formation of this ternary
complex might differ between UFH and LMWH and therefore might influence thrombin inactivation and subsequent fibrin formation. Indeed, Hogg et al found that heparins with lower molecular weight are less effective in promoting thrombin binding to fibrin polymer (48). Furthermore, during LMWH therapy, higher antithrombin levels are maintained compared to UFH (39,49,50). This implicates that in patients treated with LMWH, more thrombin will be inhibited by antithrombin and less fibrin will be generated. Although this could explain the differences in fibrin monomer levels between UFH and LMWH in our study, the increase of fibrin monomer levels in the UFH group is still not clear.

The role of heparin in the enhancement of fibrinolysis is thought to be through inhibition of thrombin-dependent generation of activated TAFI. As a result, stabilisation of a clot by TAFI will be diminished, leading to shortening of clot lysis times. In our study, we found that both heparins resulted in shorter clot lysis times after treatment. In vitro inactivation of TAFI by CPI eliminated any further effect of heparin on clot lysis times, confirming the findings of Lisman et al (51), that the effect of heparin on the clot lysis time is mainly through TAFI. However, the clot lysis times were longer in the LMWH group when compared to the UFH group (p<0.0001); this difference remained significant after inhibition of TAFI by CPI. The fact that the lysis times in our study were shorter after treatment with UFH, independent of TAFI activity, raises the question whether there are other factors involved through which UFH has more influence on clot lysis times than LMWH.

Urano et al found that addition of factor Xa together with calcium shortens clot lysis times (52). The lower anti-Xa levels in our UFH group (although not statistically significant) might therefore be the explanation for our findings. Another possibility could be the tissue-plasminogen activator (t-PA) levels. It has been shown that heparin shortens clot lysis times through an increase in t-PA (53). If UFH increases t-PA more than LMWH, this will result in shorter clot lysis times. Unfortunately, we were not able to perform these tests retrospectively.

Reports on the course of D-dimer levels during treatment with heparin and the differences between UFH and LMWH are heterogeneous (25,27,31,39,40). In our study, we found a decrease of D-dimer levels over time in both groups (p<0.0001), without differences between the groups. This implicates that LMWH has a similar effect on fibrinolysis in vivo as UFH.
As a clinical endpoint of our study, we calculated the PVOs before and at the end of treatment. There was no significant difference between the groups at day 0 and day 5. However, there was a decrease in the LMWH group, while no decrease was seen in the UFH group (p=0.04). These findings might argue in favour of LMWH as initial treatment for pulmonary embolism. From a clinical point of view, it is of interest to be informed about possible correlations between laboratory variables and perfusion abnormalities. We found a correlation between F1+2 and D-dimer levels and perfusion abnormalities, where higher levels of F1+2 or D-dimer correlated with higher vascular obstruction scores. Therefore, the initial height of F1+2 or D-dimer might give an indication of the severity of the pulmonary embolism. This is in agreement with a previous report (54). At day 5, we found no correlation between any laboratory value and perfusion abnormalities. This implicates that the coagulation and fibrinolytic markers in our study are not suitable for clinical follow-up evaluation.

In conclusion, LMWH (dalteparin) is at least equally effective as UFH in reducing coagulation activity and in prohibiting further increases in perfusion abnormalities in the early treatment of pulmonary embolism. Our findings support the use of LMWH as initial treatment of pulmonary embolism.
REFERENCE LIST


