Randomized trial of different regimens of heparins and in vivo thrombin generation in acute deep vein thrombosis

Vijay V. Kakkar, Debra A. Hoppenstead, Jawed Fareed, Zbigniew Kadziola, Mike Scully, Roumen Nakov, and Hans K. Breddin

Low-molecular-weight and unfractionated heparins are frequently used to treat venous thromboembolism, but it is not known whether they are equally effective in inhibiting in vivo generation of thrombin. In this multicenter trial, 1048 patients were randomized to intravenous unfractionated heparin (group A), twice-daily low-molecular-weight heparin (reviparmin) for 1 week (group B), or once-daily reviparin for 4 weeks (group C). All patients received vitamin K antagonists. Blood samples withdrawn at the baseline and at weeks 1 and 3 were analyzed using markers of in vivo thrombin generation and other coagulation parameters. During the first 3 weeks symptomatic recurrent deep vein thrombosis–pulmonary embolism (DVT/PE) occurred in 17 (4.5%) of 375 patients in group A compared with 4 (1.0%) of 388 patients in group B, and 9 (2.4%) of 374 patients in group C. Forty percent of patients in group A, 53.4% in group B, and 53.5% in group C showed 30% or greater reduction in thrombus size assessed by venography. Patients in group B had significantly greater reduction in D-dimer, prothrombin fragments 1 and 2 (F1 + 2), endogenous thrombin potential (ETP), and thrombin-antithrombin (TAT) complexes compared to groups A and C. Greater release of tissue factor pathway inhibitor (TFPI) and reduction in levels of thrombin activatable fibrinolysis inhibitor (TAFI) and fibrinogen were significantly more pronounced in group C patients. Reviparin administered twice daily plus vitamin K antagonist is more effective in inhibiting in vivo thrombin generation compared to intravenous unfractionated heparin plus vitamin K antagonist, and reviparin once daily produced significantly higher TFPI release and greater reduction in TAFI and fibrinogen levels. (Blood. 2002;99:1965-1970)
46 to 60 kg, and 7500 anti-Xa units for those weighing 35 to 45 kg. Also in this group treatment with a vitamin K antagonist was started on day 1. The third group of patients received LMWH (reviparin sodium) administered subcutaneously once daily (group C) for 4 weeks in the same daily weight-adjusted dosages as in group B. However, in this group, treatment with a coumarin derivative was started on day 21. In each group, treatment with vitamin K antagonists was continued for 12 weeks with the dose adjusted to maintain an international normalized ratio (INR) of 2.0-3.0.

Surveillance and follow-up

Patients were followed for 3 months in the participating centers. They were instructed to report any new symptoms that developed that were suggestive of DVT or PE, or if they developed bleeding complications. Phlebography was performed to confirm the diagnosis of recurrent DVT and ventilation-perfusion lung scanning was done for PE.

In all other patients, phlebography was performed on day 21 ± 2 to assess progression or regression of thrombus. The phleograms were assessed by an adjudication committee whose members were not aware of the treatment the patient had received and whether the phleogram was before or after the treatment. The change in thrombus size was assessed by the Marder score; the details have been previously published.17 Pulmonary emboli were diagnosed when perfusion scans demonstrated segmental or larger perfusion defects in the presence of a normal ventilation scan and a constant intraluminal filling defect or a sudden cut-off of a vessel on pulmonary angiography.

Blood samples

Venous blood was drawn in plastic syringes containing 3.8% sodium citrate from all patients on days 0, 6 ± 1, and 21 ± 2. These samples were centrifuged for 15 minutes at 2000g. Platelet-poor plasma was deep frozen and sent to a central laboratory where samples were analyzed for various coagulation parameters.

Hemostatic parameters

The aPTT was measured by a clotting time method using a commercial reagent (IL, Milan, Italy) and ACL300R Coagulometer (IL) and previously described technique.18 Anti-Xa levels were measured by amidolytic assay using a commercial kit (Coamatic Heparin, Chromogenix, Quadrache, Surrey, United Kingdom). The assay was carried out using the ACL Futura (IL). Heparin high and low controls (Sigma Diagnostics, Dorset, United Kingdom) were used as controls. Antithrombin (AT) was measured by the chromogenic amidolytic substrate method using Stachrom A T-III kits and STA analyser as described previously.19 The absorbance was measured using a microtest plate reader at 420 nm. (Dimertest Gold EAI Kit, Agen, Quadratech). Prothrombin time was measured using a microtest plate reader at 420 nm. (Hoffmann La-Roche, Basel, Switzerland). Activated factor XII levels were measured by a technique described by Ford et al,27 using a commercial kit (Shield Diagnostics, Dundee, United Kingdom), a quantitative direct enzyme immunoassay for the detection in human plasma, of activated factor XII (α-XIIa and β-XIIa). The absorbance was measured using a microtest plate reader at 550 nm.

Statistical analysis

The continuous data are expressed as median with first and third quartiles. All P values for the continuous data were computed using the Wilcoxon test for between-group comparisons or the sign test for comparisons within group. The percentages were compared by the Fisher exact test. For the relative risk (RR) the 97.5% CIs were computed. There were no adjustments for the multiple comparisons because the analysis of the hemostatic factors has been planned as an exploratory analysis (ie, hypotheses generating). All statistics were prepared with SAS v 8.2 software (SAS Institute, Cary, NC).

Results

The study included 1148 patients who were randomized. Eleven randomized patients never received study medication and were not included in the analysis. A total of 375 patients were allocated to receive UFH plus vitamin K antagonist starting on day 1 (group A), 388 patients received reviparin twice a day plus vitamin K antagonist starting on day 1 (group B), and 374 patients received reviparin once daily for 28 days with vitamin K antagonist starting on day 21 (group C). There were no relevant differences between the treatment groups with respect to the baseline characteristics (data not shown).

Symptomatic recurrent thromboembolism

During the first 3 weeks, symptomatic recurrent DVT/PE confirmed by phlebography or angiography or both occurred in 17 (4.5%) of 375 patients in group A compared to 4 (1.0%) of 388 patients in group B and 9 (2.4%) of 374 patients in group C. The recurrence rate in group A was significantly higher when compared with group B (RR, 4.40; 97.5% CI, 1.28-15.12; P = .003).

Phlebographic response rate

Evaluable phleograms were obtained at the time of inclusion and at day 21 ± 2 in 961 patients. A phlebographic responder was defined as a patient with 30% or greater reduction of the Marder score in the second phlebogram; other patients were classified as nonresponders. The responder rate was 129 (40.2%) of 321 patients in group A, 175 (53.4%) of 328 patients in group B, and 167 (53.5%) of 312 patients in group C. The nonresponse rate in group A was significantly higher than in group B (RR, 1.28; 97.5% CI, 1.08-1.52; P < .001) and higher than in group C (RR, 1.29; 97.5% CI, 1.08-1.53; P < .001).

Changes in coagulation parameters

In group A patients, intravenous infusion of UFH was adjusted to achieve aPTT to a value 1.5 to 2.5 times the baseline level. On the first day, 60.52% of patients achieved this range; on the second day, 87.71%; and on the third day, 89.58%. The INR reached the therapeutic range on day 5 in groups A and B, and on day 30 in group C, because in this group vitamin K antagonist therapy was started on day 22 only.
Results of absolute values of coagulation parameters over the 3-week period are shown in Table 1. The baseline values showed higher antifactor Xa levels in group A patients, compared with group B (P < .001) and group C (P < .001); higher TFPI levels in group A compared to group B (P < .001) and group C compared to group B (P = .01). The differences between all other coagulation parameters were not statistically significant.

At week 1, in groups A and B patients, significantly higher levels of aPPT were observed as compared to group C (P < .001). Higher antifactor Xa levels were found in groups B and C compared to group A (P < .001). A higher concentration of AT was detected in group B patients compared to group A patients (P < .001). Group B patients, compared to group A, had significantly lower levels of fibrinogen (P = .05), TAT complexes (P = .02), prothrombin F1 + 2 (P < .001), ETP (P < .001), and D-dimer (P = .06). In addition, significantly higher levels of TFPI (P = .04) and lower levels of TAFI (P = .007) were also detected in group B patients compared to group A. Comparison of groups A and C patients again showed that group A had lower levels of TAT complexes (P < .001), prothrombin F1 + 2 (P < .001), and ETP (P < .001). However, group C patients had significantly lower levels of fibrinogen (P < .001), higher AT concentrations (P < .001) and levels of TFPI (P < .001), and lower levels of TAFI (P < .001).

Comparison of values in groups B and C again showed that group B patients had significantly lower TAT complexes (P < .001), prothrombin F1 + 2 (P < .001), and ETP (P < .001). In contrast, group C patients had lower levels of fibrinogen (P < .001), higher TFPI (P < .001), and lower levels of TAFI (P < .001).

The median changes in coagulation parameters were also assessed by comparing between-group changes from time point to time point rather than between-group absolute levels. The differences between the median values at the first week and baseline as well as third week minus first week were compared (Table 2).

At week 1, significantly higher aPPT values were observed in groups A and B compared to group C (P < .001). There was a significant reduction in AT concentration in group A patients compared to groups B and C, in whom the levels, in fact, increased and the difference was statistically significant (P < .001). A significant reduction in the levels of prothrombin F1 + 2, ETP, and D-dimer was also observed in group B patients compared to groups A and C. In addition, group B patients compared to group C had a significant reduction in level of TAT complexes (P < .001). Although the levels of fibrinogen increased in each group, the

<table>
<thead>
<tr>
<th>Table 1. Intergroup comparison of the levels of coagulation parameters in groups A, B, and C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td><strong>Group A (UFH)</strong></td>
</tr>
<tr>
<td><strong>aPTT (s)</strong></td>
</tr>
<tr>
<td><strong>Ant-factor Xa (U/mL)</strong></td>
</tr>
<tr>
<td><strong>Fibrinogen (g/L)</strong></td>
</tr>
<tr>
<td><strong>AT (% of normal)</strong></td>
</tr>
<tr>
<td><strong>TAT complexes (ng/mL)</strong></td>
</tr>
<tr>
<td><strong>Prothrombin F1 + 2 (ng/mL)</strong></td>
</tr>
<tr>
<td><strong>D-dimer (ng/mL)</strong></td>
</tr>
<tr>
<td><strong>TAFI level (μg/mL)</strong></td>
</tr>
<tr>
<td><strong>Prothrombin F1 + 2 (ng/mL)</strong></td>
</tr>
<tr>
<td><strong>D-dimer (ng/mL)</strong></td>
</tr>
<tr>
<td><strong>TAFI level (μg/mL)</strong></td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td><strong>Fibrinogen (g/L)</strong></td>
</tr>
<tr>
<td><strong>TAT complexes (ng/mL)</strong></td>
</tr>
<tr>
<td><strong>Prothrombin F1 + 2 (ng/mL)</strong></td>
</tr>
<tr>
<td><strong>D-dimer (ng/mL)</strong></td>
</tr>
<tr>
<td><strong>TAFI level (μg/mL)</strong></td>
</tr>
</tbody>
</table>

Median values with first and third quartiles are shown.
increase was minimal in group C ($P < .001$). Group A patients had a significant fall in the TFPI levels compared to groups B and C ($P < .001$), in whom the levels, in fact, increased, but the difference between groups B and C was not significant. Patients in groups B and C had significantly greater reductions in the TAFI levels compared to patients in group A ($P = .006$ and .001, respectively).

At week 3, group C patients had greater fall in the levels of fibrinogen compared to groups A and B ($P = .03$ and $P < .001$, respectively). The changes in levels of TAT complexes were similar between the three groups. There was a reduction in prothrombin F1 + 2 in each group, but the change was significantly greater in group A compared to group B ($P = .03$) and group C ($P < .001$), and group B compared to C ($P < .001$). Group A patients also had greater reduction in ETP compared to group B ($P = .009$) and group C ($P = .01$). Levels of D-Dimer fell in each group but the differences were not significant. The levels of TFPI fell in each group, but the fall was significantly less in group C compared to group A ($P = .05$) and group B ($P = .001$). In groups A and B, the TAFI levels increased while in group C significant fall was observed when compared to group A and B ($P < .001$).

**Discussion**

Anticoagulant treatment for DVT aims to achieve regression of thrombi, and thus, prevent PE and recurrent thrombi. A number of trials have compared the efficacy and safety of UFH with LMWH, the primary outcome measure being a change in the size of the thrombus assessed by phlebography approximately 1 or 2 weeks after initiation of therapy.\(^1\)\(^{12}\) In recent large-scale trials, the primary outcome measure was symptomatic recurrent venous thromboembolism in patients treated with subcutaneous LMWH either hospitalized\(^12\) or at home.\(^12\)\(^{14}\) In none of these trials have blood studies been performed to determine whether treatment was effective in controlling the hypercoagulable state associated with the acute phase of venous thromboembolism.

A series of highly sensitive and specific immunochemical tools has been developed that can quantitate the levels and activities of various steps of the hemostatic mechanism in vivo at the subnanomolar level. These include prothrombin F1 + 2, which measures the cleavage of prothrombin molecule by factor Xa and TAT complexes reflecting the in vivo thrombin generation process. Recently, a method has also been developed that allows the determination of the ETP, which is a measure of the thrombin generated in defibrinated citrate plasma in vitro in response to a trigger of the extrinsic or intrinsic pathway of coagulation.\(^2\)\(^{26}\) The ETP represents the area under the thrombin generation curve, which is dependent on its concentration and time of action. The proteolytic action of plasmin on fibrin polymer cross-linked by factor XIII generates increased levels of D-dimer in plasma.\(^2\)\(^{29}\) In our study, these 4 markers of hemostatic activation were used to compare the in vivo effect of 3 anticoagulant regimens. In addition, anticoagulant response was also assessed by measuring levels of aPTT, AT, factor XIIa, TFPI activity, and fibrinogen.

The baseline values of several coagulation factors showed no significant difference except higher antifactor Xa and TFPI levels in group A patients. The intragroup comparison showed that each regimen of treatment produced significant inhibition of thrombin generation (data not shown). However, intergroup comparison showed regimen of weight-adjusted reviparin administered twice daily and combined with vitamin K antagonist (group B) was more effective in inhibiting thrombin generation than UFH plus vitamin K antagonist (group A). In addition there were highly significant differences in the in vivo markers of hemostatic activation. These changes were consistent with better clinical response in group B patients who had a lower rate of recurrent DVT/PE, 1.0% versus 4.5% (RR, 4.40; 97.5% CI, 1.28–15.12; $P = .003$), and greater reduction in thrombus size was assessed by phlebographic response rate 53.4% versus 40.2% (RR, 1.28; 97.5% CI, 1.08–1.52; $P < .001$). Although in group C patients differences in the coagulation parameters were not as pronounced as in group B, they had greater reduction in fibrinogen levels and increased release of TFPI.

The intended aim of any anticoagulant therapy is to reduce, delay, or prevent the generation of thrombin. Although the inhibitory actions of UFH and LMWHs on serine proteases are well characterized in in vitro studies, the complexities of in vivo activation of the coagulation system is that it is difficult to

### Table 2. Intergroup comparison of the changes in coagulation parameters in groups A, B, and C

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A (UFH)</th>
<th>B (reviparin bid)</th>
<th>C (reviparin od)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPTT (s)</td>
<td>15.3</td>
<td>14.3</td>
<td>2.6</td>
<td>.22</td>
</tr>
<tr>
<td>Antifactor Xa (U/mL)</td>
<td>0.06</td>
<td>0.26</td>
<td>0.19</td>
<td>.01</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>0.52</td>
<td>0.43</td>
<td>0.10</td>
<td>.17</td>
</tr>
<tr>
<td>AT (%) (of normal)</td>
<td>10</td>
<td>3.0</td>
<td>5.0</td>
<td>.001</td>
</tr>
<tr>
<td>TAT complexes (ng/mL)</td>
<td>1.4</td>
<td>1.8</td>
<td>0.82</td>
<td>.10</td>
</tr>
<tr>
<td>Prothrombin F1 + 2 (ng/mL)</td>
<td>67</td>
<td>84</td>
<td>23</td>
<td>.05</td>
</tr>
<tr>
<td>ETP-ext (mM/min)</td>
<td>264</td>
<td>330</td>
<td>297</td>
<td>.008</td>
</tr>
<tr>
<td>D-dimer (ng/mL)</td>
<td>98</td>
<td>151</td>
<td>99</td>
<td>.003</td>
</tr>
<tr>
<td>TFPI level (U/mL)</td>
<td>13</td>
<td>11.0</td>
<td>9.90</td>
<td>.006</td>
</tr>
<tr>
<td>TAFI level (µg/mL)</td>
<td>60</td>
<td>80</td>
<td>80</td>
<td>.28</td>
</tr>
</tbody>
</table>

Median values with first and third quartiles shown.
predict which reactions are most important for inhibition of thrombin generation. The situation is even further complicated by the presence of the thrombin feedback loops, whereby the traces of thrombin produced probably through activation of extrinsic pathway activate the cofactors VIII and V, which in turn produce an enormous increase in the rate and amount of thrombin subsequently generated. The anticoagulant action of UFH is primarily due to its ability to bind tightly to AT, thereby accelerating the rate of inhibition of all major coagulation enzymes. In our study in patients receiving reviparin once daily (group C), significantly higher levels of TFPI were detected both at weeks 1 and 3. In addition, these patients also had significantly lower levels of fibrinogen and TAFI. The sustained higher TFPI activity and reduced level of fibrinogen and TAFI may possibly have compensated for the reduced inhibition of thrombin generation yet significant thrombus regression observed in these patients.

This study provides further insight into the possible mechanism of action of oral anticoagulants, when combined with LMWH. In group A and B patients, therapy with vitamin K antagonists was started on day 1 and INR reached the therapeutic range in both groups by day 5, which was maintained throughout the observation period. During the first week, group B patients compared to group A had significantly lower levels of prothrombin F1 + 2, TAT complexes, ETP, and D-dimer indicating greater inhibition of thrombin generation. The conversion of prothrombin to thrombin occurs at an appreciable rate in the presence of factor Xa, factor Va, calcium ions, and platelets. Recent studies of thrombin generation indicate that relative concentration of prothrombin and AT are critical for the amount of thrombin generated. Varying these 2 proteins (in combination or individually) within their normal concentration range causes significant alteration in the amount of total thrombin available, as well as its maximum levels and rate of generation. During LMWH therapy, higher levels of AT are maintained compared to UFH. In fact, group B patients had a significantly higher concentration of AT compared to group A during week 1. The better inhibition of thrombin in group B patients may also have been facilitated by a higher AT concentration. It is possible that thrombin generation during the first week may play an important role in preventing recurrent thromboembolic disease in the subsequent weeks.

The other important finding of this study relates to the levels of TAFI. A significantly lower concentration of TAFI was observed in the group of patients who received LMWH therapy for 3 weeks. The concentration of TAFI in plasma has been reported to play an important role in the regulation of fibrinolysis. Furthermore, TAFI levels in healthy individuals correlated well with the clot lysis time and elevated TAFI levels have been shown to be a risk factor for venous thrombosis. Activation of TAFI is mediated by thrombin generated by the coagulation cascade. Experiments under in vitro and in vivo conditions have shown that in plasma an intact factor XI feedback loop is necessary to generate sufficient thrombin for TAFI activation. It is possible that LMWH is more effective than UFH in regulating the activity of this feedback loop. Recently, TAFI has also been shown to be an acute phase reactant in the mouse and human.

The results of our study indicate that a regimen of reviparin administered twice daily combined with vitamin K antagonist was more effective than UFH and vitamin K antagonist in controlling the hypercoagulable state because there were highly significant differences in the in vivo markers of hemostatic activation. These changes are consistent with better clinical response; these patients had a lower rate of recurrent DVT/PE and greater reduction in thrombus size as assessed by phlebography. Although the differences in the hemostatic markers of thrombin generation between groups B and C were also significant at weeks 1 and 3, group C patients had significantly higher levels of TFPI and lower levels of fibrinogen. It is possible that the main action of prolonged reviparin therapy in controlling the thrombotic process is due to highly significant release of TFPI and the pronounced effect of lowering fibrinogen not seen in the other 2 groups. Whether these changes are responsible for the difference in outcome between groups B and C needs further studies.

Acknowledgments

We are extremely grateful to participating centers of the CORTES Study for providing blood samples. We thank Mrs G. Dawson and Miss N. Ranall for their invaluable help in assessing various coagulation parameters and Miss Sarah Edwards in preparation of the manuscript.

References

14. The Columbus Investigators. Low molecular weight heparin is an effective and safe treatment
Randomized trial of different regimens of heparins and in vivo thrombin generation in acute deep vein thrombosis

Vijay V. Kakkar, Debra A. Hoppenstead, Jawed Fareed, Zbigniew Kadziola, Mike Scully, Roumen Nakov and Hans K. Breddin