HEPARIN KINETICS IN THROMBOEMBOLISM/Hirsh et al. 691


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Heparin Kinetics in Venous Thrombosis and Pulmonary Embolism

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SUMMARY The response to a standard dose of heparin was studied in 20 patients with venous thromboembolism. The heparin regimen consisted of intravenous injection of 70 units per kg, followed after 90 minutes by a maintenance dose of 400 units per kg per 24 hours given by continuous infusion. Plasma heparin activity and the activated partial thromboplastin time (APTT) were measured at intervals to determine clearance of the initial injection and the response to maintenance dose. Large inter-individual variations were found in the anticoagulant effect and these were due in part to differences in heparin clearance and in part to differences in the APTT response to given amounts of heparin (heparin effect index). The heparin half-life was 63 ± 15 minutes when plasma heparin activities were used for this calculation and 84 ± 71.5 minutes when the APTT was used. These results are similar to values previously reported in normal volunteers. Four of the 20 patients had pulmonary embolism and in these heparin half-life was significantly shortened (P < 0.005).

A number of studies have now demonstrated that the individual response to a standard dose of heparin differs considerably among patients with venous thromboembolism. The reason for this variation is uncertain. It could be due to inter-individual differences in heparin inactivation or clearance, to variations in the levels of circulating coagulation factors which modify the coagulation test response to heparin, or to a combination of both of these factors.

The present study was undertaken to investigate some of the mechanisms for the variation in heparin response of patients with venous thromboembolism by measuring the plasma heparin activity, the activated partial thromboplastin time (APTT), and heparin kinetics in these patients following the administration of a standard heparin dosage regimen.

Patients and Methods
Twenty patients with venous thromboembolism were studied before and during the administration of heparin. Details of age, sex and diagnosis are shown in table 1. The clinical diagnosis of venous thrombosis was confirmed by ascending venography in all but two patients, both of whom had a history of allergy to contrast medium. The diagnosis of pulmonary embolism was suspected on clinical grounds and confirmed by the results of chest X-ray and either lung

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scanning or pulmonary angiography. All patients had normal renal function established by measurement of serum creatinine and blood urea nitrogen. Liver function assessed by bilirubin, SGPT and alkaline phosphatase was normal in all patients. The concentration of total serum proteins was normal in all patients except one who had a bowel resection.

Treatment

An initial dose of 70 U/kg of aqueous sodium heparin (10,000 units per ml; M.T.C. Pharmaceuticals, Hamilton, Ontario, Canada) was given by bolus intravenous injection. After 90 minutes a maintenance dose of 400 U/kg/24 hr was given by continuous intravenous infusion using a precision syringe pump. The dose of heparin was adjusted on the basis of the result of the APTT, measured 6–15 hours after starting treatment, the aim being to maintain the APTT in the range of 60–80 sec (control 40 sec).

Many of the patients were receiving other drugs including analgesics, diuretics, digoxin, antibiotics, sedatives and steroids.

Coagulation Tests

Blood was drawn before heparin was given, at 15, 45 and 90 minutes after the bolus injection and then 6–15 hours after the maintenance infusion was started. The APTT was measured on citrated plasma prepared by centrifuging freshly drawn blood at 3000 rpm (1675 g) for 10 minutes. The platelet count in these specimens was less than 10,000/μL. Heparin activity was measured using a heparin protamine titration test with the following modifications. To 0.2 ml of test plasma was added 0.01 ml of a range of dilutions of protamine sulphate (1,000 units per ml; Connaught Medical Research Laboratories, Toronto) in veronal buffer and incubated at 37°C. After one minute 0.1 ml of thrombin (Parke Davis and Company) solution, containing 4 units per ml in isotonic saline, was added and the clotting time was recorded. Five to seven plasma samples and varying amounts of protamine were used to construct a curve relating clotting times to protamine concentrations. An in vitro heparin tolerance test was performed by measuring the APTT response of pretreatment plasma to varying concentrations of heparin over a range of 0.05 to 0.4 units per ml of plasma.

To determine the antiheparin activity of plasma, heparin in known concentrations (as indicated by the manufacturer) was added to citrated plasma and the recovery was determined by the protamine sulphate titration test. Using this test, essentially all the heparin added was recovered over a range of concentrations from 0.05 to 2.0 U/ml (table 2).

### Table 1. Heparin Half-life, Distribution Volume, Heparin Clearance and Heparin Effect Index

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>t½ heparin induced change in APTT (min)</th>
<th>Kinetics using heparin activity</th>
<th>Heparin effect index (sec/U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.M.</td>
<td>67</td>
<td>F</td>
<td>Postop. DVT; knee fracture</td>
<td>139 ± 32</td>
<td>72 ± 36</td>
<td>112.7 ± 1.23</td>
</tr>
<tr>
<td>A.H.</td>
<td>62</td>
<td>F</td>
<td>Postop. DVT; hip fracture</td>
<td>45 ± 14</td>
<td>—</td>
<td>121.8 ± 1.22</td>
</tr>
<tr>
<td>E.R.</td>
<td>65</td>
<td>M</td>
<td>Postop. DVT; hip fracture</td>
<td>45 ± 2</td>
<td>56 ± 16</td>
<td>112.8 ± 1.40</td>
</tr>
<tr>
<td>E.S.</td>
<td>52</td>
<td>F</td>
<td>Postop. DVT; calcaeneal spur</td>
<td>346 ± 10</td>
<td>88 ± 2</td>
<td>83.5 ± 0.65</td>
</tr>
<tr>
<td>J.S.</td>
<td>46</td>
<td>M</td>
<td>Postop. DVT; knee fracture</td>
<td>—</td>
<td>77 ± 22</td>
<td>121.8 ± 1.22</td>
</tr>
<tr>
<td>F.H.</td>
<td>60</td>
<td>F</td>
<td>Postop. DVT; intestinal gangrene</td>
<td>36 ± 13</td>
<td>78 ± 36</td>
<td>347.4 ± 3.08</td>
</tr>
<tr>
<td>R.N.</td>
<td>63</td>
<td>M</td>
<td>Postop. DVT; kidney transplantation</td>
<td>73 ± 34</td>
<td>57 ± 16</td>
<td>101.3 ± 1.29</td>
</tr>
<tr>
<td>L.N.</td>
<td>76</td>
<td>F</td>
<td>Postop. DVT; meningoma</td>
<td>49 ± 15</td>
<td>44 ± 27</td>
<td>55.2 ± 1.0</td>
</tr>
<tr>
<td>A.V.</td>
<td>73</td>
<td>M</td>
<td>Postop. DVT; parotid tumor</td>
<td>80 ± 34</td>
<td>58 ± 20</td>
<td>124.6 ± 1.49</td>
</tr>
<tr>
<td>B.S.</td>
<td>52</td>
<td>M</td>
<td>Postop. DVT; carcinoma of mandible</td>
<td>102 ± 4</td>
<td>54 ± 16</td>
<td>99.5 ± 1.28</td>
</tr>
<tr>
<td>A.B.</td>
<td>67</td>
<td>M</td>
<td>Postop. PE; duodenal ulcer</td>
<td>38 ± 12</td>
<td>39 ± 21</td>
<td>122.6 ± 2.16</td>
</tr>
<tr>
<td>P.Z.</td>
<td>22</td>
<td>M</td>
<td>Postop. PE and DVT; tibial fracture</td>
<td>37 ± 36</td>
<td>39 ± 13</td>
<td>125.8 ± 2.22</td>
</tr>
<tr>
<td>M.S.</td>
<td>69</td>
<td>F</td>
<td>Postop. PE; pancreatitis</td>
<td>37 ± 43</td>
<td>36 ± 46</td>
<td>210.2 ± 4.09</td>
</tr>
<tr>
<td>L.H.</td>
<td>37</td>
<td>M</td>
<td>Postop. DVT and PE; duodenal ulcer</td>
<td>99 ± 1</td>
<td>57 ± 23</td>
<td>107.9 ± 2.03</td>
</tr>
<tr>
<td>W.K.</td>
<td>60</td>
<td>M</td>
<td>DVT; chronic airway obstruction</td>
<td>104 ± 24</td>
<td>75 ± 22</td>
<td>153.2 ± 1.41</td>
</tr>
<tr>
<td>M.C.</td>
<td>27</td>
<td>F</td>
<td>DVT; pregnancy</td>
<td>66 ± 27</td>
<td>75 ± 22</td>
<td>148.2 ± 1.37</td>
</tr>
<tr>
<td>E.N.</td>
<td>56</td>
<td>F</td>
<td>DVT; chronic heart failure</td>
<td>140 ± 28</td>
<td>102 ± 5</td>
<td>126.5 ± 0.86</td>
</tr>
<tr>
<td>P.M.</td>
<td>28</td>
<td>F</td>
<td>DVT; caesarean section</td>
<td>65 ± 9</td>
<td>47 ± 4</td>
<td>91.5 ± 1.35</td>
</tr>
<tr>
<td>V.L.</td>
<td>68</td>
<td>F</td>
<td>DVT; hip fracture</td>
<td>46 ± 2</td>
<td>89 ± 19</td>
<td>66.6 ± 0.51</td>
</tr>
<tr>
<td>L.V.</td>
<td>68</td>
<td>F</td>
<td>DVT; chronic heart failure</td>
<td>56 ± 10</td>
<td>75 ± 2</td>
<td>88.8 ± 1.30</td>
</tr>
</tbody>
</table>

Mean value (± 1 sp)

84 ± 71.5 63 ± 19.2 127 ± 63 1.58 ± 0.84 156 ± 79

Abbreviations: DVT = deep venous thrombosis; PE = pulmonary embolism.

### Table 2. Recovery of Heparin Activity in Normal and Modified Plasma Using the Protamine Sulphate Titration Test and APTT

<table>
<thead>
<tr>
<th>Heparin concentration added (U/ml)</th>
<th>Test plasma</th>
<th>Heparin concentration assayed by the protamine sulphate titration test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Control plasma</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Cryoprecipitate enriched plasma</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Fibrinogen enriched** plasma</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>Diluted control plasma (1:1)</td>
<td>0</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Heparin concentration added (U/ml)

<table>
<thead>
<tr>
<th>Test plasma</th>
<th>APTT (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control plasma</td>
<td>40 100 150 233 &gt;250</td>
</tr>
<tr>
<td>Cryoprecipitate enriched plasma</td>
<td>37 38 63 83 113</td>
</tr>
<tr>
<td>Fibrinogen enriched** plasma</td>
<td>65 101 140 230 &gt;250</td>
</tr>
<tr>
<td>Diluted control plasma</td>
<td>119 210 261 &gt;250</td>
</tr>
</tbody>
</table>

*Three parts of cryoprecipitate to one part control plasma.
**Final fibrinogen concentration 1000 mg %. 
Influence of Alterations of Plasma Proteins and Clotting Factors on the APTT and the Heparin Activity of Heparinized Plasma (table 2)

To determine whether the measurement of heparin activity by the protamine sulphate titration test was influenced by the level of plasma proteins, including fibrinogen and Factor VIII, increasing concentrations of heparin were added to citrated platelet-poor plasma which was divided into four aliquots: 1) control plasma; 2) plasma to which cryoprecipitate was added to approximately double the concentrations of fibrinogen and Factor VIII; 3) plasma to which fibrinogen (human fibrinogen, Kabi) was added to double the fibrinogen concentration of the plasma; 4) plasma to which was added an equal volume of 0.85% sodium chloride in order to decrease the amount of plasma proteins and clotting factors. Heparin was added in concentrations ranging from 0.2 to 0.8 U/ml and the samples were tested with the APTT and the protamine sulphate titration test. In addition, the APTT was performed on samples before the addition of heparin.

The heparin activity measured by the protamine sulphate test was virtually identical in all four test systems while the APTT result of the heparinized plasma was markedly affected by modifying the plasma (table 2).

Calculations

The method of least squares was used to calculate a regression line for the Δ APTT* values and for the heparin activity at 15, 45 and 90 minutes after bolus injection. The half-life was calculated using both the heparin activity and the Δ APTT values; the rate of elimination, the concentration at 0 time and the volume of distribution were calculated from the plasma heparin activity. The clearance was calculated as the product of the rate constant of elimination and the volume of distribution. First order kinetics with a single compartment were assumed. The relationship between the heparin activity and the corresponding APTT was calculated by subtracting the pretreatment APTT from the APTT obtained on samples drawn after heparin infusion and dividing the result (the Δ APTT) by the heparin activity in the samples. This was called the heparin-effect index (HEI). This ratio was calculated for the samples taken at 15, 45 and 90 minutes after the bolus injection; the mean value for these three samples was taken to represent the HEI. An in vitro HEI was calculated using samples taken before heparin injection by dividing the Δ APTT by the heparin activity assayed when a range of heparin concentrations was added to the pretreatment plasma samples.

Results

Response to Bolus Injection and Maintenance Infusion

The APTT response to the intravenous bolus dose of heparin and to the maintenance heparin infusion is shown in figure 1. The pretreatment APTT varied from 22 to 46 seconds (mean 34 seconds) and the post heparin response varied widely from patient to patient. The plasma heparin activity after the bolus injection and during the maintenance infusion is shown in figure 2. Again, there was a marked

*Δ APTT = APTT after heparin injection – pretreatment APTT.
and the APTT response in the individual patients is reflected in the HEI. In figure 4, the HEI is plotted for each patient at 15, 45 and 90 minutes after heparin injection. Although the HEI at each of these times after bolus injection is similar in each patient, there is a wide variation in HEI among the various patients, the index-value ranging from 65 to 342 sec/U/ml.

The in vitro HEI obtained by adding heparin to pre-treatment plasma showed a strong correlation \((r = 0.87)\) with the HEI observed after heparin administration, a finding which suggests that the factors responsible for the in vitro anticoagulant effect obtained in any particular patient were present in the patient's plasma before heparin treatment.

Heparin Kinetics

Heparin kinetics were calculated in 18 of the 20 patients (table 1). In two patients there were insufficient samples to calculate heparin clearance. The mean heparin clearance was 1.58 ± 0.84 \((\text{SD})\) ml/kg/min and the heparin half-life was 63 ± 19 min when the plasma heparin activity was used for this calculation. The half-life of the heparin-induced change in APTT was 84 ± 71.5 min. There was no effect of age or sex on either the heparin clearance or the HEI. Because the patients in the study were being treated with a variety of other drugs, it was impossible to determine whether these drugs influenced heparin kinetics.

The four patients with pulmonary embolism had a significantly greater heparin clearance and a significantly shorter heparin half-life \((P < 0.005)\) than those with venous thrombosis (table 3). Patients with pulmonary embolism had a lower hepatic level at 15 minutes (fig. 2) than those with venous thrombosis but the difference between the two groups was not statistically significant. There was no difference in the HEI between the patients with pulmonary embolism and venous thrombosis. Thus, the tendency for the APTT to be lower in patients with pulmonary embolism, shown in figure 2, is due to differences in the heparin activity reached rather than to a lesser APTT response to a given amount of heparin.

None of the patients developed clinical evidence of recurrent thromboembolism nor did they develop clinically significant bleeding.

**Discussion**

This study has demonstrated that the large inter-individual variation in the anticoagulant response to heparin in patients with venous thromboembolism is due in part to differences in clearance and in part to differences in the APTT response to a given amount of heparin, i.e., in the HEI. Clearance of heparin is influenced by the level of circulating anti-heparin activity and by the rate of hepatic inac-
tivation and renal clearance of heparin. Liver and renal function was normal in our patients but this does not exclude the possibility that variations in heparin metabolism by the liver or kidneys could have contributed to the differences in the heparin kinetic results.

The reason for the variation between individuals in the anticoagulant response to heparin (HEI) is not entirely clear, but could have been due in part to differences in the level of coagulation factors and plasma proteins. We have shown that the addition, in vitro, of fibrinogen and cryoprecipitate (Factor VIII), as well as dilution of normal plasma, markedly influences the APTT result in heparinized plasma without affecting the heparin activity determined by the protamine sulphate titration method.

The results of heparin kinetic data in our patients with venous thrombosis were similar to those reported by Estes in normal volunteers. However, the range of results in our patients with venous thromboembolism was considerably wider than that reported in the normal volunteers. Furthermore, the standard deviation of the clearance of heparin activity using the APTT results was much greater than that of the heparin activity detected by the protamine sulphate titration method. It is likely that this difference is due partly to the imprecision of the APTT test when it is prolonged to more than 100 seconds by heparin. However, using either the ΔAPTT or the heparin activity there was a significantly shorter heparin half-life and greater heparin clearance in the four patients with pulmonary embolism than in patients with venous thrombosis. Although the numbers were small, the differences observed between patients with pulmonary embolism and venous thrombosis were highly significant.

The mechanism of the increased heparin clearance observed in acute pulmonary embolism is uncertain but it may be related to continuing thrombin formation on the surface of the embolus. Thomas and associates reported that experimental pulmonary emboli became rapidly coated with platelets but that experimental venous thrombi remained relatively free of coated platelets. This difference in the degree of platelet adhesion to venous thrombi and pulmonary emboli could be caused by differences in the rate of thrombin generation on the respective surfaces. If more thrombin was formed on the surface of pulmonary emboli, it could lead to an increased rate of heparin clearance either through release of anti-heparin activity from platelets exposed to thrombin or, through complexing of the heparin-antithrombin complex, to thrombin and other activated clotting factors that are generated on the surface of the embolus. Irrespective of the mechanism, the finding of increased heparin clearance in acute pulmonary embolism supports the suggestion that large doses of heparin should be given initially to patients with acute pulmonary embolism.

Acknowledgment

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References

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