Highly Variable Anticoagulant Response After Subcutaneous Administration of High-Dose (12,500 IU) Heparin in Patients With Myocardial Infarction and Healthy Volunteers

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Background. In this study, the anticoagulant response of 12,500 IU heparin s.c. was investigated in patients with myocardial infarction and healthy volunteers to determine variabilities in response and modifying factors.

Methods and Results. On the fourth day after thrombolytic therapy, blood samples were taken before and at frequent intervals until 10 hours after the injection of 12,500 IU heparin s.c. Plasma anti-Xa activity, anti-IIa activity, and the activated partial thromboplastin time (APTT) were measured in addition to body weight and thickness of the abdominal subcutaneous fat layer. Contrary to expectations, the increase of anti-Xa activity, anti-IIa activity, and APTT compared with baseline (predrug) levels was very small, with an average maximal APTT of 42.6 seconds (SD, 12.4 seconds; range, 30.4–70.7 seconds). Subsequently, the influence of the length of the injection needle on the anticoagulant effect of 12,500 IU heparin s.c. was studied in 10 healthy volunteers to find a factor that could be responsible for the poor response in the patients. The length of the injection needle did not influence the anticoagulant effect of heparin. Large interindividual and intraindividual variabilities were seen in the volunteers. The majority of volunteers had minimal prolongation of the APTT, but very strong prolongation was also seen (maximal APTT, 163 seconds). There was no correlation between the abdominal skinfold thickness and anti-Xa activity, anti-IIa activity, or APTT (p>0.05), but in the patient study, there was a correlation between weight and anti-Xa activity and anti-IIa activity (p<0.05), and in the volunteer study, there was a correlation between weight and anti-Xa activity and APTT (p<0.05).

Conclusions. Subcutaneous administration of heparin in a fixed dose for prophylactic and therapeutic purposes may be inadequate because of the large interindividual and intraindividual variations in anticoagulant effect. (Circulation 1992;86:1370–1375)

Key Words • absorption • thrombosis • pharmacokinetics • heparin

Heparin is a widely used antithrombotic drug for the treatment and prevention of thromboembolic disorders.1–3 Originally, heparin was given intravenously in a dose adjusted to its anticoagulant effect for therapeutic purposes and in a fixed subcutaneous dose for prophylaxis of deep venous thrombosis. More recently, the subcutaneous administration of heparin in both fixed and adjusted doses was evaluated in situations where therapeutic anticoagulation used to be employed.4,5 Heparin currently is also administered subcutaneously after myocardial infarction for prevention of reocclusion after thrombolysis or prevention of left ventricular thrombi.6–8 The more convenient administration of heparin in fixed dosage by the subcutaneous route may be used more extensively in the future. However, some doubt was raised regarding this route of administration since the absorption of heparin was highly variable9 and in part dependent on abdominal skinfold thickness,10 at least in healthy volunteers under standard conditions.

To investigate this further, we studied the anticoagulant response of heparin after subcutaneous administration (12,500 IU) to patients with suspected or definite acute myocardial infarction. In addition, the effect of the length of the injection needle on the absorption of subcutaneous heparin was studied in healthy volunteers to investigate another factor that could influence the absorption of subcutaneous heparin.

Methods

Subjects and Protocol

Part 1. Eight patients with suspected or definite acute myocardial infarction who participated in the Third
International Study of Infarct Survival (ISIS-3)\textsuperscript{11} gave informed consent for the study, which was approved by the Ethical Review Board of Leiden University Hospital. Patients were excluded if they had taken any oral anticoagulant drug before entering the ISIS-III protocol or if they had reduced peripheral circulation due to heart failure. Baseline variables of the patients are shown in Table 1. Patients received 12,500 IU calcium heparin (Calparine, Sanofi) in the lateral abdominal wall with a 25-g (0.5×16 mm) needle according to the ISIS-III protocol after fibrinolytic therapy with streptokinase, tissue-type plasminogen activator (t-PA), or APSAC. On the fourth day after thrombolytic therapy, blood samples were taken immediately before and at intervals after the subcutaneous injection of 12,500 IU heparin.

Venous blood for the determination of plasma anti-Xa activity, anti-IIa activity, and activated partial thromboplastin time (APTT) was collected in \( \frac{1}{10} \) vol of 0.11 M trisodium citrate. Platelet-poor plasma was obtained by centrifugation of the blood for 5 minutes at 6,000g and stored at \(-40^\circ\text{C}\).

Part 2. Ten, nonsmoking, healthy male volunteers gave informed consent for the study, which had been approved by the Ethical Review Board of Leiden University Hospital. They were between 20 and 27 years old, and their weight ranged from 66 to 105 kg. The thickness of their abdominal fat layer at the site of the injection was between 10 and 40 mm. They were not taking any other drugs, and consumption of alcohol was stopped 48 hours before and for the duration of each treatment occasion. None of the subjects had a condition known to be associated with an increased bleeding risk (e.g., hypertension, gastrointestinal erosions or ulcers, use of antiplatelet drugs, or acquired or congenital hemorrhagic diathesis).

The study was an open, randomized, crossover trial in which subjects received two treatments in random order with a washout period of 1 week. Treatment A was an subcutaneous injection of 12,500 IU calcium heparin (Calparine, Sanofi) lateral in the abdominal wall given with a 23-g (0.6×25 mm) needle. Treatment B consisted of the same dose given with a 25-g (0.5×16 mm) needle, which was the size used for the patients. A standard technique was used for both injections, which were always given by the same physician. A skinfold was raised, and the needle was inserted at a right angle to the skin surface. The injection was given slowly, and the needle was withdrawn gradually, after which the skin was released. The calcium heparin was supplied by the pharmacy of Leiden University Hospital and was obtained from one batch.

Blood samples were taken immediately before and at frequent intervals after the subcutaneous injection. Venous blood for the determination of plasma anti-Xa activity, anti-IIa activity, and APTT was collected in \( \frac{1}{10} \) vol of 0.11 M trisodium citrate. Platelet-poor plasma was obtained by centrifugation of the blood for 5 minutes at 6,000g and stored at \(-40^\circ\text{C}\).

Subjects remained at the clinical research center until the last blood sample was taken.

### Analytical Methods

Chromogenic amidolytic assays were used to measure plasma anti-Xa and anti-IIa activities. These assays were performed on an ACL Automated Coagulation Laboratory Analyzer (Instrumentation Laboratory, Milan, Italy). The plasma anti-Xa activity assay was performed with the Coatest heparin kit (Kabi Vitrum, Stockholm, Sweden) according to Teien et al\textsuperscript{12} with slight modifications. The within-day and between-day CV values were approximately 4.4\% (n=19) and 5.6\% (n=90), respectively, at a concentration of 0.4 IU/ml. The detection limit was 0.05 IU/ml. For the plasma anti-IIa activity assay according to Larsen et al,\textsuperscript{13} the IL test heparin kit (Instrumentation Laboratory) was used. The within-day and between-day CV values were approximately 3\% (n=19) and 4\% (n=90), respectively, at a concentration of 0.6 IU/ml. The detection limit was 0.05 IU/ml. The APTT assay was performed on an ACL Automated Coagulation Laboratory Analyzer with automated APTT reagent (General Diagnostics Inc., N.J.) according to standard techniques.\textsuperscript{14} Normal values ranged from 26 to 40 seconds.

Body weight and abdominal subcutaneous fat were measured by a blinded observer. Skinfold thickness was measured with Harpenden skinfold caliper (British Indicators Ltd., Bedfordshire, U.K.) and by ultrasound (Toshiba 250 echo-Doppler instrument with a 5-MHz transducer) in the volunteer study.
Pharmacokinetic Analysis

The area under the curve (AUC) was calculated for the APTT, anti-Xa activity, and anti-IIa activity up to the last sampling point (AUC₀₋₅) by linear trapezoidal approximation after subtraction of baseline (predrug) activity values.

The maximum values of the APTT (APTTₘₐₓ) and anti-Xa activity (anti-Xₐₐₓ) were taken from the data by visual inspection. Calculations were carried out using the SIPHAR software package (Simed, Creteil, France).

Statistical Analysis

Because the study results were highly skewed, the statistical analysis was carried out using nonparametric tests. All tests were two-tailed, and significance values were set at 5%. For comparison of the effects induced by the different needles, the Wilcoxon matched-pairs signed-ranks test was used. Weight-related parameters were correlated with parameters representing the magnitude of absorption of heparin using Spearman's rank correlations. All calculations were carried out using the spss/pc+V4.0.1 statistical software package (SPSS, Inc., Chicago, Ill.).

Results

Part 1

The individual and mean APTT and anti-Xa activity values versus time curves are shown in Figure 1. This demonstrates that virtually no increase in APTT occurred in the patients. This was also the case for the anti-IIa activity, which is not shown graphically.

The individual AUC values of plasma anti-Xa activity, anti-IIa activity, APTT, anti-Xₐₐₓ, and APTTₘₐₓ are presented in Table 2. One patient had a high anti-Xa activity and a low APTT. Only one of the patients had an APTTₘₐₓ that was 1.5-fold that of the control value at any time, but this patient also had a high predose value. Two of the patients had an anti-Xₐₐₓ value of more than 0.2 IU/ml, but these patients also had high predose values. There was no correlation between skinfold thickness and the AUC of the anti-Xa activity, anti-IIa activity, APTT, anti-Xₐₐₓ, or APTTₘₐₓ (p > 0.05). There was also no correlation between weight and the AUC of the APTT, APTTₘₐₓ, or anti-Xₐₐₓ (p > 0.05). There was a significant negative correlation between weight and AUC of the anti-Xa activity (r = -0.71, p = 0.05) and between weight and AUC of the anti-IIa activity (r = -0.90, p = 0.002).

Part 2

There was no significant difference in AUC of APTT, plasma anti-IIa activity, and anti-Xa activity between the two routes of administration (p > 0.05) nor was this the case for APTTₘₐₓ and anti-Xₐₐₓ (p > 0.05). The average APTT and anti-Xa curves for both treatments are shown in Figure 2. The mean APTTₘₐₓ for treatment A was 64.3 (SD, 34.3) and for treatment B was 60.8 (SD, 37.2), but one subject had a very high APTTₘₐₓ value of 163 seconds (approximately fourfold the baseline value). The individual AUCs of plasma anti-Xa, anti-IIa activity, APTT, anti-Xₐₐₓ, and APTTₘₐₓ are presented in Table 3. Two volunteers had a high anti-Xa activity but a low APTT. There was no difference in abdominal skinfold thickness as measured by the Harpenden caliper or ultrasound. There was no correlation between skinfold thickness and AUC of anti-Xa activity, anti-IIa activity, APTT, APTTₘₐₓ, anti-Xₐₐₓ for both treatments (p > 0.05).

Discussion

In contrast with expectations from our recent volunteer study and previous investigations, only a minimal anticoagulant response could be measured in the patients by anti-IIa activity, anti-Xa activity, and APTT after subcutaneous administration of 12,500 IU heparin. Only one patient was adequately anticoagulated (APTTₘₐₓ, 72 seconds; Figure 1), but this patient had a higher baseline value, resulting in a net increase in anticoagulation that was just as minimal as that in the other patients.

It cannot be excluded that the patients had an increased heparin clearance after myocardial infarction or that ab-
TABLE 2. Area Under the APTT, Anti-IIa, and Anti-Xa Curves and APTT_{max} of Eight Patients After Subcutaneous Administration of 12,500 IU Heparin

<table>
<thead>
<tr>
<th>Patient</th>
<th>AUC APTT (sec · min/10^4)</th>
<th>AUC anti-IIa (min · IU/ml)</th>
<th>AUC anti-Xa (min · IU/ml)</th>
<th>APTT_{max} (seconds)</th>
<th>Anti-Xa_{max} (IU/ml)</th>
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<td>22.0</td>
<td>24.2</td>
<td>12.4</td>
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AUC, area under the curve; APTT, activated partial thromboplastin time.

**FIGURE 2.** Panel A: Plot of APTT clotting time-time profiles (mean±SD) in 10 subjects after s.c. administration of 12,500 IU heparin with a 23-g needle (○) and a 25-g needle (△). Panel B: Plot of anti-Xa activity–time profiles (mean±SD) in 10 subjects after s.c. administration of 12,500 IU heparin with a 23-g needle (○) and a 25-g needle (△).
TABLE 3. Area Under the APTT, Anti-IIa, and Anti-Xa Curves and APTT\textsubscript{max} and Anti-Xa\textsubscript{max} of 10 Subjects After Subcutaneous Administration of 12,500 IU Heparin With a 23-g Needle and With a 25-g Needle

<table>
<thead>
<tr>
<th>Subject</th>
<th>AUC APTT (sec \cdot min/10^3)</th>
<th>AUC anti-IIa (min \cdot IU/ml)</th>
<th>AUC anti-Xa (min \cdot IU/ml)</th>
<th>APTT\textsubscript{max} (seconds)</th>
<th>Anti-Xa\textsubscript{max} (IU/ml)</th>
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<td>19.1</td>
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<td>176.8</td>
<td>122.7</td>
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<td>102.4</td>
<td>95.6</td>
<td>141</td>
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<td>72.8</td>
<td>73.3</td>
<td>80.1</td>
<td>75.7</td>
</tr>
<tr>
<td>SD</td>
<td>60.6</td>
<td>68.2</td>
<td>34.4</td>
<td>44.9</td>
<td>56.9</td>
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</table>

AUC, area under the curve; APTT, activated partial thromboplastin time; A, use of 23-g needle; B, use of 25-g needle.

in other conditions such as proximal vein thrombosis, in which subcutaneous heparin is used as an alternative to intravenous heparin.\textsuperscript{4}

The conclusions from the large fibrinolytic trials on myocardial infarction (ISIS-III\textsuperscript{11} and Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico [GISSI-2]\textsuperscript{8}) was that the benefits of high-dose s.c. heparin did not clearly outweigh the risks, since in these studies only a small decrease in reinfarction was nearly compensated for by an increased occurrence of bleeding.

FIGURE 3. Panel A: Plot of individual APTT clotting time–time profiles and mean value (heavy line) in 10 subjects after s.c. administration of 12,500 IU heparin with a 23-g needle. Panel B: Plot of individual anti-Xa activity–time profiles and mean value (heavy line) in 10 subjects after s.c. administration of 12,500 IU heparin with a 23-g needle.

FIGURE 4. Panel A: Plot of individual APTT clotting time–time profiles and mean value (heavy line) in 10 subjects after s.c. administration of 12,500 IU heparin with a 25-g needle. Panel B: Plot of individual anti-Xa activity–time profiles and mean value (heavy line) in 10 subjects after s.c. administration of 12,500 IU heparin with a 25-g needle.
These results were surprising because thrombolysis exposes a thrombogenic surface in the coronary artery, and therefore additional anticoagulation should help prevent reocclusion. Furthermore, intravenous heparin has a beneficial effect in other acute coronary syndromes, such as unstable angina. If the patients in the present study are representative of the study population of these large trials, the level of anticoagulation may have been insufficient. However, even if relatively stable anticoagulation can be obtained by the use of intravenous heparin, the desired level of anticoagulation after fibrinolysis is not well known. The relative value of the more convenient subcutaneous heparin regimen and intravenous heparin as an adjunct therapy to thrombolysis will be addressed in the ongoing GUSTO trial.

We did not find a correlation between abdominal skinfold thickness and anti-Xa activity, anti-IIa activity, and APTT, but in the patient study there was a high correlation between weight and AUC of anti-Xa activity and anti-IIa activity, and in the volunteer study there was a high correlation between weight and AUC of anti-Xa activity and anti-Xa activity max for treatment A and between weight and APTT for both treatments. The effects of weight on the kinetics of heparin have been described previously, and the negative relation may be related to the larger distribution volume of the heavier patients. The lack of a relation between skin thickness and the anticoagulant response found in this study is not necessarily in disagreement with previous data. The anticoagulant response after heparin was poor; furthermore, we did not formally study the bioavailability of heparin, which would have required comparison with an intravenous dose. In addition to the considerable interindividual variation in anti-Xa activity, anti-IIa activity, and APTT after subcutaneous heparin administration, as has been demonstrated by others previously, we found a large intraindividual variation in anticoagulant response in this study, which is in contrast with a previous study in which a reproducible anticoagulant effect was seen in subjects after repeated subcutaneous administration of heparin. This indicates that in addition to weight, other factors may influence the anticoagulant response of subcutaneous heparin.

The benefit-to-risk ratio of subcutaneous heparin may be improved by adjusted dosing as suggested previously by Turpie and colleagues. The maximum effect in anticoagulation occurred approximately 3 hours after administration of the subcutaneous heparin. This study demonstrates that the timing of the determination of the APTT is crucial when subcutaneous dosage is adjusted on the basis of this measurement. In addition, in this study, anticoagulant measurements were followed during the day, demonstrating that if a twice-daily dosing schedule were used, anticoagulant control would be minimal at the end of the day and presumably early in the morning, when the risk of infarction may be greater.

In conclusion, subcutaneous administration of a fixed high dose of heparin may be inadequate to obtain therapeutic or prophylactic levels of anticoagulation or, in view of the extremely high APTT values in some of the volunteers, possibly hazardous.

The anticoagulant response after subcutaneous heparin is very variable and dependent on weight and other unknown local factors, which may induce excessive interindividual and intraindividual variability. Subcutaneous heparin should be administered in adjusted doses according to weight and its anticoagulant effect, but this may be difficult because large day-to-day variability may occur.

References

Highly variable anticoagulant response after subcutaneous administration of high-dose (12,500 IU) heparin in patients with myocardial infarction and healthy volunteers. C Kroon, W R ten Hove, A de Boer, J M Kroon, J M van der Pol, E J Harthoorn-Lasthuizen, H C Schoemaker, F J van der Meer and A F Cohen

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