Pharmacodynamics and Pharmacokinetics of Dermatan Sulfate in Humans

By F. Dol, G. Houin, M. Rostin, J.L. Montastruc, D. Dupouy, F. Gianese, P. Sie, and B. Boneu

Dermatan sulfate (DS), a catalyst of the thrombin-heparin cofactor II interaction, has antithrombotic activity and is devoid of significant hemorrhagic risk in several animal models. We investigated the pharmacodynamic and pharmacokinetic properties of DS in humans. DS was injected in single bolus intravenous injections of four increasing doses (0.5, 1, 1.5, 2 mg/kg) to six healthy volunteers. The resulting anticoagulant activities were assessed by the activated partial thromboplastin time (APTT) and the thrombin clotting time (TCT). There were dose-dependent prolongations of the APTT and TCT, and the anticoagulant activities disappeared in less than three hours. The pharmacokinetic parameters were calculated from the plasma concentrations of DS measured with a new chromogenic assay. The volume of distribution was ~1.8 times greater than the theoretical plasma volume and was independent of dose. In contrast, the clearance decreased with dose and the terminal half-life ranged from 0.45 ± 0.08 hours at 0.5 mg/kg to 0.72 ± 0.11 hours (mean ± SD) at 2 mg/kg. The bioavailabilities of subcutaneous (SC) and intramuscular (IM) administration relative to those of intravenous administration were determined in 12 other volunteers. The respective bioavailabilities were 24.7% ± 12.9% and 12.4% ± 9.2% for SC and IM administration. There was no detectable change in the APTT and the TCT when the volunteers were injected with 1.5 mg/kg SC or IM. In addition, the pharmacokinetic parameters derived from plasma concentrations of DS showed considerable interindividual variations by the two later routes of administration. Peak concentrations were noted 2.7 ± 1.3 hours after SC injection and 4.3 ± 4.9 hours after IM injection. The average peak concentrations were 0.7 ± 0.3 and 0.4 ± 0.2 mg/L after SC and IM injections, respectively. The half-lives of DS were 7.5 ± 6.5 hours (SC) and 6.3 ± 7.4 hours (IM). No adverse reaction to DS was recorded during this study.

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The principle of the assay involves estimation of the residual described in detail previously. In the present work, we adapted this DOL human thrombin was obtained from the Centre National de Trans-purified from human plasma as already described and semi-purified the reaction cup 3 seconds before simultaneous addition of the containing 1% polyethylene glycol, [PEG]) was then dispensed into The procedure was performed at 37°C. Fifty microbiters of human automate. Subsequent sample handling was completely automated. of a 100 mg/mL Bentonite (Sigma, St Louis) suspension in Tris 0.02 amidolytic thrombin activity after a short incubation of thrombin was measured at 405 nm for 10 seconds. At a DS level of 1 sg/mL, dispensed in the reaction cup and the mixture was transferred substrates (CBS 34-47, purchased from Stago (Asnières, France) 0.5 mmol/L in Tris 0.02 mol/L (pH 7.4), NaCl 0.3 mol/L) was was added, and the residual amidolytic activity was determined Fig 2. Typical calibration curve for dermatan sulfate assay in plasma. Increased amounts of dermatan sulfate were dissolved in control plasma before defibrination. A given amount of thrombin was added, and the residual amidolytic activity was determined after an incubation time of 55 seconds (described in the Materials and Methods section).
dual method between the first and the last measurable experimental
dose (0.5 mg/kg) was eliminated faster than the three higher
detectable antifactor Xa activity 5, 15, and 30 minutes after
analysis.

or to the method of administration were performed with variance
of the pharmacokinetic parameters according to the delivered dose
comparison with IV administration were calculated as the ratios of
the respective AUC

and TCT were dose dependent, and coagulation times
returned to the preinjection values between 1 and 3 hours
after injection, depending on the injected dose. There was no
detectable antifactor Xa activity 5, 15, and 30 minutes after
the injections.

Figure 3 shows the decrease of DS concentrations in the
plasma. The elimination curves were monoexponential and
were roughly parallel for the three higher doses. The phar-
cokinetic parameters derived from these curves are sum-
marized in Table 2. There were excellent linear correlations
between the delivered doses and the Cmax, whereas after IV administration the anticoagulant
activities were similar to those observed in the first part of
this study after IV injection of the same dose.

Figure 4 shows the profile of the plasma concentrations of
DS with time for each subject. There were large interindivid-
al variations for both methods of administration. The
observed Cmax ranged from 0.33 to 1.2 µg/mL after SC
injection and from 0 to 0.85 µg/mL after IM injection. In
many subjects, the appearance of DS in plasma with time
was irregular. Therefore, no attempt was made to model the
pharmacokinetic pattern, and area under the plasma concen-
tration curve extrapolated to infinity was not calculated.

Pharmacokinetics after IV injection. The anticoagulant
activities generated by the IV injections of four increasing
doses of DS are shown in Table 1. Prolongations of the APTT
and TCT were dose dependent, and coagulation times
returned to the preinjection values between 1 and 3 hours
after injection, depending on the injected dose. There was no
detectable antifactor Xa activity 5, 15, and 30 minutes after
the injections.

Table 1. Anticoagulant Activities (in Seconds) Generated After IV Injection of Four Increasing Doses of Dermatan Sulfate to Six Healthy Subjects

<table>
<thead>
<tr>
<th>Test</th>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>5</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
</tr>
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<tbody>
<tr>
<td>APTT</td>
<td>0.5</td>
<td>33 ± 4</td>
<td>41 ± 3</td>
<td>38 ± 5</td>
<td>35 ± 4</td>
<td>33 ± 3</td>
<td>33 ± 6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>33 ± 5</td>
<td>49 ± 8</td>
<td>42 ± 7</td>
<td>34 ± 3</td>
<td>35 ± 3</td>
<td>31 ± 3</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>32 ± 4</td>
<td>50 ± 5</td>
<td>46 ± 7</td>
<td>39 ± 4</td>
<td>35 ± 3</td>
<td>33 ± 2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>33 ± 5</td>
<td>53 ± 8</td>
<td>47 ± 4</td>
<td>45 ± 5</td>
<td>38 ± 4</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>TCT</td>
<td>0.5</td>
<td>17 ± 1</td>
<td>26 ± 2</td>
<td>20 ± 1</td>
<td>19 ± 1</td>
<td>18 ± 1</td>
<td>18 ± 1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>17 ± 1</td>
<td>108 ± 74</td>
<td>31 ± 7</td>
<td>21 ± 2</td>
<td>18 ± 1</td>
<td>17 ± 1</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>17 ± 1</td>
<td>138 ± 70</td>
<td>44 ± 25</td>
<td>27 ± 7</td>
<td>19 ± 2</td>
<td>18 ± 2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17 ± 1</td>
<td>&gt;200</td>
<td>122 ± 86</td>
<td>43 ± 27</td>
<td>21 ± 3</td>
<td>19 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

RESULTS

No side effects were recorded during the study, except that
some volunteers indicated a slight pain at the IM injection
site at the moment of injection or within two hours after it.
Routine biochemical and hematologic parameters remained
unchanged at the end of the study.

Bioavailabilities of DS administered IM and SC. The
bioavailabilities of IM and SC administrations were investi-
gated after a 1.5-mg/kg injection. When DS was delivered
IM and SC, there were no detectable changes in the APTT or
TCT, whereas after IV administration the anticoagulant
activities were similar to those observed in the first part of
this study after IV injection of the same dose.

Fig 3. Disappearance of plasma dermatan sulfate concentra-
tions (mean and SD) after IV bolus injection of four increasing
doses to six healthy subjects. The four lines correspond to 0.5, 1,
1.5, and 2 mg/kg, respectively (bottom to top).
Figure 5 shows the mean DS plasma concentrations obtained with the three routes of administration, and Table 3 summarizes the pharmacokinetic parameters derived from individual curves. The $C_{\text{max}}$ and the area under the plasma concentration curve extrapolated to infinity after IV injection were higher than those observed in the first series of volunteers, but the mean $t_{1/2}$ of disappearance was essentially the same. The plasma DS peak concentrations after SC and IM injections were reached 2.7 and 4.3 hours after injection, respectively, and these activities disappeared with $t_{1/2}$ of 7.9 and 6.3 hours on the average. As compared with IV administration, the mean availabilities were 24.7% and 12.4%, respectively, for SC and IM administration.

**DISCUSSION**

A pharmacokinetic study of DS is a prerequisite to further pharmacologic developments of this compound as an antithrombotic agent. To monitor the concentration of the drug in the plasmas of normal human volunteers, we used a new, sensitive, and convenient chromogenic assay based on the catalytic effect of DS on thrombin–HC II interaction. A comparison of the APTT and TCT prolongations (Table 1) with the evolution of plasma concentrations of DS after IV injection (Fig 1) suggests that no detectable alteration occurred in these tests at concentrations <2 to 4 μg/mL. This observation was confirmed by the IM and SC studies. The chromogenic assay appears to be at least 20 to 40 times more sensitive for estimating the concentration of DS than the APTT or the TCT. It is thus suitable for pharmacokinetic studies after IM or SC administration of DS.

IV injection of four increasing doses of DS to the same volunteers allowed us to determine how the dose influenced the pharmacokinetic parameters and to compare the observed parameters with those previously reported for UH and low-molecular-weight heparins (LMWH). The Vd of DS was higher than the theoretical plasma volume and was independent of the administered dose. In contrast, the clearance was significantly reduced and the $t_{1/2}$ of disappearance was prolonged with increasing dose. The overall profile is comparable to that reported for UH and suggests a saturable mechanism of clearance for both DS and heparin. However, the changes of the pharmacokinetic parameters of DS noted with increasing doses were less pronounced than those reported for UH. In the report of Olsson et al, when healthy volunteers received 100, 200, and 400 IU/kg, the respective $t_{1/2}$ of disappearance was 56, 96, and 152 minutes. In the report of Bjornsson et al, the respective $t_{1/2}$ after injection of 25, 50, and 75 IU/kg to volunteers was 28, 39, and 48 minutes, and the corresponding total clearance...
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Fig 5. Plasma concentration curves (mean and SD) of dermatan sulfate generated after IV (△), SC (●), or IM (○) injection of 1.5 mg/kg to 12 healthy subjects.

was 8.2, 5.6, and 4.6 L/h. In contrast to these marked changes observed with UH, the pharmacokinetic parameters of DS, at least in the range of the doses tested, were only marginally influenced by dose. These properties are therefore close to those of LMWH, for which no dose dependency was observed.14,18 This may explain why the profile of plasma DS disappearance was not concave-convex as reported for unfractionated UH.16,17

The $t_1/2$ of disappearance of DS after IV injection was consistently shorter than that reported for UH after injection of equivalent doses on a weight basis.12,14,20,22 In these human studies, UH was injected at doses ranging from 0.16 to 4 mg/kg and the $t_1/2$ of disappearance ranged from 0.46 to 2.5 hours. However, these pharmacokinetic data were derived from the biologic activities (antifactor IIa or antifactor Xa) of heparin: These activities are linked to the heparin moiety which binds with high affinity to antithrombin III, (ie, about one third of heparin species23,24), and cleared at a slower rate than the low-affinity fraction.25,26

In contrast, the mean rate of DS elimination after SC injection was ~3.5 and 2.0 times slower than those of heparin and LMWHs, respectively.27-30 From this pattern, after repeated SC or IM administrations of MF 701, a steady-state level can be expected, consistently higher than the peak concentrations obtained in this study after a single administration. The mean availabilities were 12.4% and 24.7% for IM and SC routes, respectively. These values, probably underestimated since the AUC generated after IM and SC injection were not extrapolated to infinity, were considerably lower than those reported for LMWHs.27-30 There are at least two possible reasons for these differences. First, absorption of DS from the sites of injection and its diffusion into the intravascular compartment could be incomplete, as a direct result of its relatively higher molecular weight.31 Second, the lower plasma concentrations generated by the slow rate of absorption from the injection site are cleared very quickly. In support of this hypothesis, the IV studies indicate that low doses of DS are eliminated faster than high doses. Further investigations are required to distinguish between these hypotheses. These results were obtained after a DS injection of 1.5 mg/kg; investigating SC or IM DS availabilities after injection of higher doses will be of interest.

### Table 3. Pharmacokinetic Parameters of Dermatan Sulfate After IV, SC, and IM Injections of 1.5 mg/kg to 12 Healthy Subjects

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>IV</th>
<th>SC</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CINF (mg/L)</td>
<td>25.2 ± 5.8</td>
<td>0.67 ± 0.28</td>
<td>0.41 ± 0.25</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>2.70 ± 1.30</td>
<td>4.30 ± 4.90</td>
<td></td>
</tr>
<tr>
<td>AUC (mg/L/h)</td>
<td>22.1 ± 6.3</td>
<td>5.10 ± 2.50</td>
<td>2.70 ± 2.10</td>
</tr>
<tr>
<td>AUC extr. (mg/L/h)</td>
<td>24.5 ± 6.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>$t_1/2$ (h)</td>
<td>0.65 ± 0.12</td>
<td>7.89 ± 6.50</td>
<td>6.26 ± 7.41</td>
</tr>
<tr>
<td>F (%)</td>
<td>24.7 ± 12.9</td>
<td>12.4 ± 9.20</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ND, not determined; AUC extr., area under the plasma concentration curve extrapolated to infinity.

Values are mean ± SD.

REFERENCES

27. Dawes J, Bara L, Billaud E, Samama M: Relationship between biological activity and concentration of a low-molecular-weight heparin (PK 10169) and unfractionated heparin after intravenous and subcutaneous administration. Haemostasis 16:116, 1986
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