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 7.9 ± 8.5 hours (SC) and 6.3 ± 7.4 hours (IM). No adverse reaction to DS was recorded during this study.

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Human thrombin was obtained from the Centre National de Trans-

purified from human plasma as already described and semi-purified

sample (100 μL) and thrombin (100 μL, 3 NIH U/mL in Tris 0.02

containing 1% polyethylene glycol, [PEGJ) was then dispensed into

The procedure was performed at 37°C. Fifty microliters of human

amidolytic thrombin activity after a short incubation of thrombin

method to the automate SBA 300 (Corning-Gilford, Oberlin, OH).

was measured at 405 nm for 10 seconds. At a DS level of 1 μg/mL,

was calculated by viscosimetry using K and a values 1.01

and

weight determinations were

performed with reference

standards

curves

with DS

samples whose molecular weight

was calculated by viscosimetry using K and a values 1.01 and 1.7 × 10^{-3}, respectively.22

Assay of DS. The method for measuring DS in plasma was

described in detail previously.7 In the present work, we adapted this

method to the automate SBA 300 (Corning-Gilford, Oberlin, OH).

The principle of the assay involves estimation of the residual

amidolytic thrombin activity after a short incubation of thrombin

with heparin cofactor II (HC II) in defibrinated plasma containing

DS.

One volume of citrated platelet-poor plasma was mixed with 1 vol

of a 100 mg/mL Bentonite (Sigma, St Louis) suspension in Tris 0.02

mol/L (pH 7.4) buffer. After strong mixing, the tubes were cen-

trifuged and the supernatant was placed in the sample cup ring of the

automate. Subsequent sample handling was completely automated.

The procedure was performed at 37°C. Fifty microliters of human

purified HC II (1 μmol/L in Tris 0.02 mol/L [pH 7.4] buffer containing

1% polyethylene glycol, [PEGJ) was then dispensed into the reaction cup 3 seconds before simultaneous addition of the

sample (100 μL) and thrombin (100 μL, 3 NIH U/mL in Tris 0.02

mol/L [pH 7.4] 0.075 mol/L NaCl, 1% PEG buffer). HC II was

purified from human plasma as already described8 and semi-purified

human thrombin was obtained from the Centre National de Trans-

fusion Sanguine (Paris). After 55 seconds, 200 μL chromogenic

substrate (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

dispensed in the reaction cup and the mixture was transferred

immediately to the spectrophotometer cuvette. The amidolytic rate

was measured at 405 nm for 10 seconds. At a DS level of 1 μg/mL,

the intraassay and interassay variabilities were 11% and 16%,

respectively.

A standard curve was used for each series of measurements, made

up of the samples obtained from each volunteer before administra-

tion of each dose by each route. Standard curves were obtained by

adding known amounts of MF 701 (0, 0.5, 1, 1.5, and 2 μg/mL, final

concentrations before bentonite absorption) to autologous plasma

sampled in each volunteer before DS injection. As shown in Fig 2,

there was a linear relationship between the DS concentrations and

the log of change in absorbance per minute which varied from 0.38 ±

0.01 at 0 μg/mL DS to 0.126 ± 0.01 at 2 μg/mL DS (mean ± SEM,

n = 60). DS concentrations in the samples were calculated from the

appropriate standard curve. Plasmas containing more than 2 μg/mL

DS (most of the samples obtained after IV administration) were

diluted before defibrination by the autologous plasma. At least two

different dilutions were performed so that final DS concentration

was in the range of 0.5 to 2 μg/mL. In most cases, DS concentrations

calculated from the two dilutions differed <15%. When the differ-

ence was greater, a third dilution was performed. In each case, the

mean values were used. Plasma sampled after SC or IM administra-

tion were directly assayed in duplicate.

Anticoagulant activities. The DS anticoagulant activities were

assessed by using the APTT (APTT reagent, Organon Teknika,

Durham, NC) and the TCT. The TCT was performed with human

thrombin (Fibrindex, Orthodiagnostic) diluted so that the control

plasma clotted between 17 and 19 seconds. These tests were

performed with a KC 4 automate (AHS-Dade, Cergy Pontoise,

France).

Anti-factor Xa activity was determined 5, 15, and 30 minutes

after IV injection. This activity was assayed with a chromogenic

assay (Stachrom heparin, Stago) using unfractionated heparin for

the calibration curve.

Calculation of the pharmacokinetic parameters. The pharma-

cokinetic parameters of DS after IV injection were estimated

according to a single open-compartmental model using the nonlinear

Marquardt algorithm for curve fitting.9 Data of individual volun-

teers were analyzed separately. The following usual parameters were

calculated; maximal concentration at time zero (C_{max}), elimination

half-life (t_{1/2}), area under the plasma concentration curve extrapo-

lated to infinity (AUC extr.), clearance of elimination (Cl), distribu-

tion volume (Vd), and mean residence time (MRT).

In view of the low circulating plasma concentrations and the

interindividual variations, no specific modelization was performed

after SC and IM injection. The major pharmacokinetic parameters

obtained were as follows: The observed values were taken for

maximum plasma concentrations (C_{max}) and the corresponding

times (T_{max}); the areas under curves were calculated by the trapezoi-

Fig 1. Chromatographic profiles of MF 701 in HPLC (top) and

PAGE (bottom). The HPLC experiments were performed with

Protein Pak 300 sw and Protein Pak 125 columns and ultraviolet

detection (203 nm); retention times are indicated in minutes.

PAGE plates were stained with toluidine blue. The molecular

weight determinations were performed with reference to standard

curves obtained with DS samples whose molecular weight

was calculated by viscosimetry using K and a values 1.01 and 1.7 × 10^{-3}, respectively.

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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).
the first and the last measurable experimental points (AUC t); terminal t/2 was calculated by the least-squares method; the availabilities (F) of IM and SC administration in comparison with IV administration were calculated as the ratios of the respective AUC t and expressed in percentage. The comparisons of the pharmacokinetic parameters according to the delivered dose or to the method of administration were performed with variance analysis.

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.

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.

Figure 3 shows the decrease of DS concentrations in the plasma. The elimination curves were monoeponential and were roughly parallel for the three higher doses. The pharmacokinetic parameters derived from these curves are summarized in Table 2. There were excellent linear correlations between the delivered doses and the Cmax (r = .99), or the AUC extrapolated (r = .99). However, the results obtained at the dose of 1.5 mg/kg were underestimated by 25% as compared with the theoretical values calculated from the regression lines. The volumes of distribution were significantly higher than the theoretical plasma volumes of this population (2.64 ± 0.60 l, t < .001, paired t test) calculated from body weight, height, and hematocrit, and were independent of the dose delivered. There was a progressive reduction of the clearance when the dose delivered increased (r = -.98). Consequently, the t/2 of disappearance and the mean residence times significantly increased with the dose (P < .001). The statistical analysis for each elimination parameter (Newman-Keul's test) showed that the lowest dose (0.5 mg/kg) was eliminated faster than the three higher doses. No significant differences were observed between the three higher doses.

Bioavailabilities of DS administered IM and SC. The bioavailabilities of IM and SC administrations were investigated 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.

Figure 4 shows the profile of the plasma concentrations of DS with time for each subject. There were large interindividual 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 concentration curve extrapolated to infinity was not calculated.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Time (min)</th>
<th>Values are mean ± SD.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>APTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>33 ± 4</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>1</td>
<td>33 ± 5</td>
<td>49 ± 8</td>
</tr>
<tr>
<td>1.5</td>
<td>32 ± 4</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>2</td>
<td>33 ± 5</td>
<td>53 ± 8</td>
</tr>
<tr>
<td>TCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>17 ± 1</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>1</td>
<td>17 ± 1</td>
<td>108 ± 74</td>
</tr>
<tr>
<td>1.5</td>
<td>17 ± 1</td>
<td>138 ± 70</td>
</tr>
<tr>
<td>2</td>
<td>17 ± 1</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

Fig 3. Disappearance of plasma dermatan sulfate concentrations (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).
**Table 2. Pharmacokinetic Parameters of Dermatan Sulfate After IV Injection of Four Increasing Doses to Six Healthy Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Delivered Doses (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Cmax (mg/L)</td>
<td>6.80 ± 1.60</td>
</tr>
<tr>
<td>AUC extr. (mg/L/h)</td>
<td>4.49 ± 0.91</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>0.45 ± 0.08</td>
</tr>
<tr>
<td>Cl (L/h)</td>
<td>6.98 ± 1.69</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>4.45 ± 0.97</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.65 ± 0.12</td>
</tr>
</tbody>
</table>

Abbreviation: AUCextr., area under the plasma concentration curve extrapolated to infinity.

Values are mean ± SD.

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 Cmax 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 t½ 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 t½ 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 plasma 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.7 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 t½ of disappearance was prolonged with increasing dose. The overall profile is comparable to that reported for UH12–14 and suggests a saturable mechanism of clearance for both DS and heparin.15–17 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,1 when healthy volunteers received 100, 200, and 400 IU/kg, the respective t½ of disappearance was 56, 96, and 152 minutes.13 In the report of Björnsson et al,13 the respective t½ 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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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.\textsuperscript{14,18,19} This may explain why the profile of plasma DS disappearance was not concave-convex as reported for unfractionated UH.\textsuperscript{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.\textsuperscript{12,14,20,21} 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 species\textsuperscript{22,23}), and cleared at a slower rate than the low-affinity fraction.\textsuperscript{15,16}

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.\textsuperscript{7,30} 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.\textsuperscript{37,30} 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.\textsuperscript{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.

REFERENCES

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>Parameter</th>
<th>IV</th>
<th>SC</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (mg/L)</td>
<td>25.2 ± 5.8</td>
<td>0.67 ± 0.28</td>
<td>0.41 ± 0.25</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>—</td>
<td>2.70 ± 1.30</td>
<td>4.30 ± 4.90</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>—</td>
<td>24.7 ± 12.9</td>
<td>12.4 ± 9.20</td>
</tr>
</tbody>
</table>

Values are mean ± SD.


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