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 ± 6.5 hours (SC) and 6.3 ± 7.4 hours (IM). No adverse reaction to DS was recorded during this study.

Study design. Eighteen healthy volunteers (eight men and ten women, age range 20 to 35 years) gave their free informed consent to participate in this study. A 20-gauge Teflon catheter was placed into an antecubital vein to allow serial blood sampling. Citrated blood samples (vacutainers BD, Ref. 676608) were obtained 10 minutes before and immediately before DS injections. In the first part of the study, six volunteers received four doses of DS (0.5, 1, 1.5, and 2 mg/kg) in a vein of the opposite arm, with 1 week between injections and in random order. In the second part of the study, 12 additional volunteers received 1.5 mg/kg of DS, by IV, IM, and SC route according to a randomized crossover administration. After DS injection, 11 to 13 citrated blood samples were obtained in 24 hours. These samples were centrifuged immediately (3,000 g for 15 minutes at room temperature), and the platelet-poor plasmas were stored at −30°C until assayed. These protocols were approved by the ethical committee of the Midi Pyrénées region.

MATERIALS AND METHODS

DS. DS (MF 701, batch 01/701/C), extracted from pig intestinal mucosa, was provided by Mediobanum Farmaceutici (Milan, Italy). DS migrates in acid buffers as a single electrophoretic band, without detectable contamination of heparin, heparan sulfate, or chondroitin sulfate. Its apparent average molecular weight, determined by high-performance liquid chromatography (HPLC) was 35,700, with 80% of the product in the range of 15,000 to 45,000 and by polyacrylamide gel electrophoresis (PAGE) was 28,000 (range 8,000 to 45,000). The different molecular weight values determined by HPLC and PAGE (Fig 1) do not necessarily imply discrepancies between data obtained with the two methods. Whereas migration in HPLC is essentially a function of the size of the gel pores, in PAGE it depends on both the porous size of the gel and the degree of sulfation of the compound. The ratio of sulfate to carboxyl, determined by conductimetry, was 1.22, and the specific optical rotation (α)25 was −68.3 degrees. DS had a specific antithrombin activity of 2 U/mg and was devoid of any antifactor Xa activity as determined in human plasma using unfractionated heparin (UH) as a standard and specific chromogenic assays. For IV and IM administration, DS was prepared in injectable ampoules containing 100 mg MF 701 in 2 mL saline solution (a concentration of 50 mg/mL); for the SC route, ampoules containing 100 mg MF 701 in 0.5 mL were used (a concentration of 200 mg/mL). Both MF 701 preparations are stable at room temperature for at least 2 years.

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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 1578

human thrombin was obtained from the Centre National de Trans-

purified from human plasma as already described and semi-purified

time before simultaneous addition of the reaction cup 3 seconds before simultaneous addition of the

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

automate. Subsequent sample handling was completely automated.

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

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 sg/mL,

dispensed in the reaction cup and the mixture was transferred

substrate (CBS 34-47, purchased from Stago (Asnières, France) 0.5

was calculated by viscosimetry using K and a values 1 .01 and 1.7 x

obtained were as follows: The

the pharma-

calculated’#{176}: maximal concentration at time zero (C,,..), elimination

tears were analyzed separately. The following usual parameters were

estimated according to a single open-compartmental model using the nonlinear

Marquardt algorithm for curve fitting. Data of individual volun-

ters were analyzed separately. The following usual parameters were calculated, maximal concentration at time zero (C,,..), elimination

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-

the areas under curves were calculated by the trapezoi-

Assay of DS. The method for measuring DS in plasma was
described in detail previously. 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

diluted before defibrination. A given amount of thrombin

After strong mixing, the tubes were centri-

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

were analyzed separately. The following usual parameters were

calculated 4: maximal concentration at time zero (C,,..), elimination

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 difference was greater, a third dilution was performed. In each case, the mean values were used. Plasma sampled after SC or IM administration 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-

the areas under curves were calculated by the trapezoi-

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 (Tmax); the areas under curves were calculated by the trapezoi-
Pharmacokinetics of Dermatan Sulfate in Humans

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>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>APTT</td>
<td>0.5</td>
<td>33 ± 4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>33 ± 5</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>32 ± 4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>TCT</td>
<td>0.5</td>
<td>17 ± 1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>17 ± 1</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>17 ± 1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17 ± 1</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

dal method between the first and the last measurable experimental points (AUC t); terminal $t_{1/2}$ 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 monoexponential 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 $C_{\text{max}}$ ($r = .99$), 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, P < .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 $t_{1/2}$ 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 $C_{\text{max}}$ 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.

![Figure 3](https://example.com/figure3.png)

**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></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>2</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>CI (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: AUC extr., 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 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 t½ 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 t½ of disappearance was 56, 96, and 152 minutes. In the report of Björnsson et al, 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...
PHARMACOKINETICS OF DERMATAN SULFATE IN HUMANS

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. This may explain why the profile of plasma DS disappearance was not concave-convex as reported for unfractionated UH.  

The 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.  

In these human studies, UH was injected at doses ranging from 0.16 to 4 mg/kg and the 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), and cleared at a slower rate than the low-affinity fraction.

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. 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. 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. 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>Pharmacokinetic Parameter</th>
<th>IV</th>
<th>SC</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (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>6.2 ± 1.3</td>
<td>4.3 ± 0.90</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>t1/2 (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.
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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