Pharmacokinetic and Antithrombotic Properties of Two Pentasaccharides With High Affinity to Antithrombin III in the Rabbit: Comparison With CY216

By D. Carrie, C. Caranobe, S. Saivin, G. Houin, M. Petitou, J.C. Lormeau, C. Van Boeckel, D. Meuleman, and B. Boneu

This study compares the pharmacokinetic and the antithrombotic properties of two pentasaccharides with high affinity to antithrombin III with those of a conventional low molecular weight heparin, CY216, in the rabbit. On a weight basis, SR 90107A/ORG 31540 (natural pentasaccharide [NPS]) and SR 80027A/ORG 31550 (sulfated pentasaccharide [SPS]) were, respectively, 4.7 and 26 times more potent anti-factor Xa inhibitory agents than CY216. They were devoid of antithrombin activity, whereas the anti-factor Xa/antithrombin ratio of CY216 was 3.8. After bolus intravenous administration, the clearance (mL/kg/h) of CY216 decreased from 31 ± 27 for the dose of 12.5 U/kg to 49 ± 14 for the dose of 50 U/kg and then remained constant up to the highest dose tested (500 U/kg). The clearance of NPS was unrelated to the dose and comparable to that of CY216 over 50 U/kg, whereas that of SPS was 10 times lower. Consistent results were observed after continuous intravenous infusions for 9 hours and subcutaneous administration. The duration of the antithrombotic effect was compared after a single subcutaneous injection of 250 U/kg of either compound in the stasis-Wessler model using human serum as thrombogenic stimulus. Two hours after the injection, the three compounds provided a thrombus prevention of greater than 95% and mean plasma activities of 0.8, 0.8, and 1.9 U/ml for CY216, NPS, and SPS, respectively. Twelve hours after injection, the antithrombotic effects of CY216 and NPS had totally vanished, whereas that of SPS was 68%. At that time, the plasma anti-Xa activities were less than 0.06 U/ml for CY216 and NPS, but 1.1 U/ml for SPS. For the latter compound, significant antithrombotic effects and detectable anti-Xa activities were still recorded 48 hours after the injection. The antithrombotic potency of the three compounds was also compared as their ability to inhibit the growth of a standardized venous thrombosis during 4 hours. The lowest total doses providing the maximum inhibitory effect were 3,125, 1,428, and 62 μg/kg for CY216, NPS, and SPS, respectively. These doses generated mean steady state antifactor Xa activities of 1.06, 1.5, and 1.2 anti-Xa U/ml, respectively. These observations indicate that the amplification mechanisms triggered by thrombin bound to fibrin and leading to the generation of new thrombin are essential to ensure venous thrombosis growth and that these mechanisms may be efficiently inhibited by pure anti-factor Xa targeting agents.

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LOW MOLECULAR WEIGHT heparins (LMWHs) currently used for therapeutic purposes are prepared from unfractionated heparin with various procedures. This accounts for the different proportions of polysaccharide chains having a high affinity to antithrombin III (ATIII) and a molecular weight greater than 5,400 Daltons contained in each preparation. Above this molecular weight, heparin chains catalyze the inhibition of both factor Xa and factor IIa by ATIII, whereas below this molecular weight, only factor Xa is inhibited. The combination of these two properties governs the specific activities and the antifactor Xa/antifactor IIa ratio of any LMWH.

After parenteral administration, the disappearance of polysaccharide chains is influenced by their affinity to ATIII and their molecular weight. Materials having a low affinity to ATIII and/or the highest molecular weight are cleared faster than those with high affinity and low molecular weight. Thus, the composition of an LMWH changes after parenteral injection; its antifactor Xa/antifactor IIa ratio progressively increases due to a faster elimination of the antifactor IIa activity. Despite this structural and pharmacodynamic heterogeneity, we have recently reported that several LMWHs, having increasing antifactor Xa/antifactor IIa ratios ranging from 1.7 to 6.8, were equipotent to inhibit venous thrombus growth when delivered on the basis of equivalent international antifactor Xa units to the rabbit. However, in these experiments, it was difficult to correlate the antithrombotic effect to any heparin subfractions or activities.

The pentasaccharide structure that allows the binding of heparin to specific sites of ATIII molecules has been synthesized. This molecule exerts a pure anti-Xa effect and is devoid of antifactor IIa activity at relevant plasma concentrations. A number of pentasaccharide analogues differing from the natural pentasaccharide (NPS) have also been produced. They differ from NPS by their sulfate content or position, their affinity to ATIII, and their specific antifactor Xa activity. These well-defined molecules offer the possibility of better understanding the mechanism of the antithrombotic action of an LMWH and, more specifically, of determining the relative contribution of the antifactor Xa inhibitory effect. In the present study, we compare the pharmacokinetics, the pharmacodynamic profile, and the antithrombotic potency of a conventional LMWH (Fraxiparine or CY216) with those of two pentasaccharides with high affinity to ATIII.

MATERIALS AND METHODS

Heparins. Heparins were provided by Sanofi-Recherche (Gentilly, France) and by Organon International (Oss, The Netherlands).

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CY216 (batch P533 XH) was prepared from unfractionated heparin of porcine mucosal source by deamination cleavage followed by an ethanol fractionation step. SR 90107A/ORG 31540, or NPS, was synthesized as previously reported.* SR 90107NORG 31540, or NPS, was devoid of any detectable antithrombin activity in these ATIII-dependent assays. SPS was prepared from unfractionated heparin of human serum used in these experiments. The results observed in treated animals were compared to those of saline-treated animals. The wet thrombi were weighed and the antithrombotic effect was expressed as the percentage of inhibition by reference to the weight of thrombi obtained in saline-treated animals (n = 26). The antithrombotic effect of each compound at each time point was tested on groups of 6 to 8 animals.

### Table 1. Molecular Weights and Relative Specific Activities in Rabbit Plasma of CY216, NPS, and SPS

<table>
<thead>
<tr>
<th>Heparin</th>
<th>Molecular Weight (Dalton)</th>
<th>Relative Specific Activities (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CY216</td>
<td>4,500 (mean)</td>
<td>Anti-Xa 80, Anti-IIa 21</td>
</tr>
<tr>
<td>NPS</td>
<td>1,728</td>
<td>Anti-Xa 300, Anti-IIa &lt;0.06</td>
</tr>
<tr>
<td>SPS</td>
<td>1,830</td>
<td>Anti-Xa 2,000, Anti-IIa &lt;0.06</td>
</tr>
</tbody>
</table>

### Table 2. Pharmacokinetic Parameters of CY216, NPS, and SPS After Bolus IV Injection of Increasing Doses (Anti-Xa U/kg) in Rabbits

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (U/kg)</th>
<th>Cmax (U/mL)</th>
<th>t1/2 (h)</th>
<th>Vd (mL/kg)</th>
<th>AUC (U/mLh)</th>
<th>CI (mL/kg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CY216</td>
<td>12.5</td>
<td>0.24 ± 0.03</td>
<td>0.4 ± 0.1</td>
<td>50 ± 5</td>
<td>0.14 ± 0.04</td>
<td>91 ± 27</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.54 ± 0.09</td>
<td>0.4 ± 0.2</td>
<td>37 ± 12</td>
<td>0.4 ± 0.1</td>
<td>76 ± 31</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>1.11 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>46 ± 13</td>
<td>1.1 ± 0.3</td>
<td>49 ± 14</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.8 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>63 ± 20</td>
<td>2.1 ± 0.2</td>
<td>49 ± 6</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>3.6 ± 0.6</td>
<td>1.4 ± 0.4</td>
<td>76 ± 19</td>
<td>6.8 ± 1.1</td>
<td>38 ± 6</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5.6 ± 0.8</td>
<td>2.1 ± 0.4</td>
<td>139 ± 24</td>
<td>11.2 ± 1.1</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>NPS</td>
<td>12.5</td>
<td>0.21 ± 0.03</td>
<td>1.1 ± 0.6</td>
<td>80 ± 27</td>
<td>0.26 ± 0.07</td>
<td>54 ± 19</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.38 ± 0.07</td>
<td>3.1 ± 1.3</td>
<td>116 ± 21</td>
<td>0.96 ± 0.3</td>
<td>31 ± 12</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.64 ± 0.05</td>
<td>2.6 ± 1.9</td>
<td>124 ± 18</td>
<td>1.5 ± 0.9</td>
<td>46 ± 29</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.4 ± 0.2</td>
<td>1.9 ± 0.8</td>
<td>120 ± 47</td>
<td>2.4 ± 0.8</td>
<td>46 ± 14</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>3.2 ± 0.9</td>
<td>2.7 ± 1.1</td>
<td>162 ± 28</td>
<td>5.8 ± 1.6</td>
<td>46 ± 14</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>5.8 ± 0.9</td>
<td>2.3 ± 0.2</td>
<td>184 ± 42</td>
<td>9.4 ± 1.7</td>
<td>54 ± 10</td>
</tr>
<tr>
<td>SPS</td>
<td>25</td>
<td>0.55 ± 0.07</td>
<td>16 ± 7</td>
<td>96 ± 6</td>
<td>6 ± 2</td>
<td>4.6 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>1.1 ± 0.1</td>
<td>17 ± 7</td>
<td>95 ± 24</td>
<td>13 ± 3</td>
<td>4.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.4 ± 0.2</td>
<td>24 ± 18</td>
<td>106 ± 33</td>
<td>29 ± 13</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>5.5 ± 0.7</td>
<td>20 ± 13</td>
<td>115 ± 56</td>
<td>57 ± 15</td>
<td>4.8 ± 1.7</td>
</tr>
</tbody>
</table>

Results are given as mean ± SD of 6 to 8 animals.
comparison with saline-treated animals. The details of the experimental procedure have been reported recently.\textsuperscript{11}

Saline and heparins were delivered under continuous IV infusion (0.5 mL/h) via the marginal ear vein. For CY216 and NPS, the doses ranged from 5 to 250 anti-Xa U/kg/h. To reach steady state plasma concentrations rapidly, these continuous infusions were preceded by bolus intra-arterial injections of the same doses (5 to 250 U/kg). For SPS, the very low clearance of elimination was taken in account. The bolus doses were comparable to those of CY216 or NPS, but the doses delivered during the 4-hour infusion period were considerably lower (see Table 6). Arterial blood samples were taken 30, 60, 120, and 240 minutes after starting the infusion and the steady state plasma activities were calculated as the mean of the four values. The antithrombotic activities of each compound and dose were evaluated on 8 to 10 animals.

\textit{Determination of the anticoagulant activities.} Arterial blood samples were processed as indicated above. The anticoagulant activities were evaluated by the activated partial thromboplastin time (APTT; Actin FS; Dade, Aguada, Puerto-Rico). Heparin concentrations were determined with ATIII-dependent antifactor Xa and antifactor IIa chromogenic assays, adapted onto the SBA 300 Gilford automate (Ciba-Corning, Cergy-Pontoise, France). The antifactor Xa activity was determined using a commercial kit (Stachrom Heparin; Stago, Asnières, France) according to the specifications of the manufacturer. The antithrombin activity was measured by determining the residual activity of exogenous thrombin added to dilute plasma in presence of bovine ATIII on the chromogenic substrate S 2238 (Kabi, Montpellier, France). The technical details of the methods have been reported elsewhere.\textsuperscript{4} The results were expressed in antifactor Xa units or antifactor IIa units per milliliter using calibration curves constructed with a pool of rabbit platelet-poor plasma and the heparins injected to the animal. The lowest sensitivity of these assays was 0.05 U/mL.

\textit{Statistical analysis.} The results of the pharmacokinetic experiments are given as mean ± SD. The influence of the dose on the clearance of elimination was tested using variance analysis followed by a Neuman-Keuls test if necessary. The comparison of the clearance of the three heparins was made by a variance analysis test. The results of the antithrombotic experiments were given as mean ± SEM. The percentages of thrombus prevention (Wessler model) or of inhibition of fibrin accretion (venous thrombosis growth model) of treated animals was compared with those of the controls using a Mann and Whitney test. The relative potencies of the antithrombotic effects of CY216 and of the two pentasaccharides were compared using a variance analysis test with the log-dose or log-plasma anti-Xa activities as covariants. These statistical analyses were performed with the PCSM computer program (Deltasoft, Meylan, France). Differences having a P value <.05 were considered significant.

\begin{table}[h]
\begin{center}
\begin{tabular}{|c|c|c|c|}
\hline
& \textbf{C} & \textbf{AUC} \\
\hline
\textbf{Dose} & \textbf{(anti-Xa U/mL)} & \textbf{Cl} & \textbf{(ml/kg/h)} \\
\hline
\textbf{CY216} & 0.22 ± 0.05 & 1.13 ± 0.19 & 45 ± 8  \\
\textbf{NPS}   & 0.16 ± 0.05 & 0.86 ± 0.18 & 72 ± 11  \\
\textbf{SPS} & 0.67 ± 0.01\textsuperscript{*} & 2.9 ± 0.2 & NC  \\
\hline
\end{tabular}
\caption{Ces and Cl Obtained After A 9-Hour Continuous IV Infusion of CY216, NPS, or SPS at Two Different Doses}
\end{center}
\end{table}

\textbf{RESULTS}

\textit{Pharmacokinetic experiments.} The results of the pharmacokinetic experiments are summarized in Tables 2 through 4. After bolus IV administration, the mean $C_{\text{max}}$ and AUC of the three compounds were related to the doses injected ($r > .99$). The volume of distribution of CY216 and of NPS progressively increased with the dose as a consequence of a distribution phase becoming more apparent at the higher doses. This phenomenon was more important for NPS than for CY216. The clearance (mL/kg/h) of CY216 significantly decreased from 91 ± 27 at the dose of 12.5 U/kg to 49 ± 14 at the dose of 50 U/kg ($P < .001$) and then exhibited nonsignificant variations until the highest dose tested. The clearance of NPS remained constant and, on average, slightly higher than that of CY216 over 50 U/kg (nonsignificant difference). Pharmacokinetic parameters (half-life and volume of distribution) of SPS were also constant regardless of the dose delivered, but the clearance was 10 times lower than those of CY216 and NPS. Figure 1 illustrates the different behavior of the three compounds after a bolus IV injection of 250 U/kg.

The results obtained after constant IV infusion and subcutaneous administration were consistent with those obtained after bolus IV injection. Due to a very low clearance of elimination, the plateau of concentrations generated by the constant infusion of the same dose was considerably higher with SPS than with CY216 and NPS (Table 3). It is noteworthy that, because of the very long half-life of SPS (14 to 20 hours), the steady state concentrations had not been achieved 9 hours after starting the infusion, so the clearance was not calculated. After subcutaneous administration (Table 4), the $C_{\text{max}}$ was reached 2 to 3 hours after the injection and the half-lives were 3.8 ± 0.9 hours and 3.1 ± 0.7 hours for CY216 and NPS, respectively, but 24 ± 7 hours for SPS. The bioavailabilities were close to 100%. The lower bioavailability calculated for SPS probably results from uncertainties in the calculation of the AUCs due to the very long half-life of this compound.

\textit{Duration of the antithrombotic effect after single subcutaneous administration.} The three compounds were delivered subcutaneously at the dose of 250 U/kg and the resultant antithrombotic effect was tested 2 hours ($C_{\text{max}}$), 12 hours, and 24 hours after the injection. For SPS, another group of animals was also tested 48 hours after the injection. The results of these experiments are summarized in Table 5. Two hours after the injection, the thrombus prevention was greater than 90% for the three compounds. At that time, plasma antifactor Xa activities were comparable for CY216 and NPS, but almost two times higher for SPS. The antithrombotic activity of CY216 and NPS disappeared in less than

\begin{table}[h]
\begin{center}
\begin{tabular}{|c|c|c|c|}
\hline
& \textbf{C} & \textbf{AUC} \\
\hline
\textbf{Dose} & \textbf{(anti-Xa U/mL)} & \textbf{Cl} & \textbf{(ml/kg/h)} \\
\hline
\textbf{CY216} & 0.22 ± 0.05 & 1.13 ± 0.19 & 45 ± 8  \\
\textbf{NPS}   & 0.16 ± 0.05 & 0.86 ± 0.18 & 72 ± 11  \\
\textbf{SPS} & 0.67 ± 0.01\textsuperscript{*} & 2.9 ± 0.2 & NC  \\
\hline
\end{tabular}
\caption{Css and Cl Obtained After A 9-Hour Continuous IV Infusion of CY216, NPS, or SPS at Two Different Doses}
\end{center}
\end{table}
12 hours after the injection; the antifactor Xa activity was either very low (0.06 U/mL) or below the limit of sensitivity of our assays. In contrast, for SPS, residual significant antithrombotic and detectable antifactor Xa activities were observed until 48 hours after the injection.

Inhibition of venous thrombosis growth. The antithrombotic potencies of the three heparins were evaluated as their ability to inhibit venous thrombosis growth during a 4-hour continuous IV infusion. The experiments were designed to generate antifactor Xa steady state concentrations of the same order of magnitude. The dose regimens used are summarized in Table 6. For CY216, the maximum inhibitory effect (75%) was obtained for mean plasma anti-Xa and anti-IIa concentrations of 1.06 and 0.21 U/mL, respectively. Increasing by 5 times the dose delivered and by 7 to 8 times the resultant plasma concentrations did not improve the results. For NPS and SPS, the maximum antithrombotic effects (65% to 68%) were slightly lower than that of CY216 (nonsignificant difference) and were obtained for antifactor Xa plasma concentrations of 1.2 to 1.5 U/mL. For SPS, increasing by 3 times the anti-Xa plasma concentration did not improve the antithrombotic effect. Figure 2 shows the relationship between the antithrombotic effect and the total dose injected expressed on a gravimetric basis. Due to its high specific activity associated to a low clearance, SPS was considerably more potent than NPS and than CY216. Figure 2 also shows the relationship between the antithrombotic effect and the resultant antifactor Xa plasma concentrations. The curves of NPS and of SPS were almost superimposable but not parallel with that of CY216.

DISCUSSION

The main objective of this study was to compare the antithrombotic effects of CY216, an LMWH having an antifactor Xa/antifactor IIa ratio of 3.8, with those of two SPSs having a pure antifactor Xa effect. SR 80027A/ORG 31550 (or SPS) differed from SR 90107A/ORG 31540 (or NPS) by only one sulfate residue in position 3 of the H sugar unit. This resulted in a reduction of the kd of the pentasaccharide/rabbit AT III complex from 132 to 6 nmol/L,15 an increased anti-Xa specific activity, and a dramatic prolongation of the terminal half-life after bolus IV administration to the rats.16 To properly design the dose-regimens used in these experiments, it was first necessary to characterize the pharmacokinetic properties of these three compounds. After bolus IV injection, the clearance of CY216 based on antifactor Xa activity significantly decreased from 12.5 to 50 U/kg and then remained constant up to the dose of 500 U/kg. This twofold

Table 4. Pharmacokinetic Parameters of CY216, NPS, and SPS After Subcutaneous Injection of 500 Anti-Xa U/kg

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Cmax (U/mL)</th>
<th>t/2 (h)</th>
<th>AUC (U/mLh)</th>
<th>CI (mL/kg/h)</th>
<th>Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CY216</td>
<td>1.7 ± 0.2</td>
<td>3.8 ± 0.9</td>
<td>14.8 ± 2.5</td>
<td>34 ± 7</td>
<td>132</td>
</tr>
<tr>
<td>NPS</td>
<td>1.5 ± 0.1</td>
<td>3.1 ± 0.7</td>
<td>9.4 ± 2.2</td>
<td>55 ± 12</td>
<td>100</td>
</tr>
<tr>
<td>SPS</td>
<td>3.5 ± 0.3</td>
<td>24 ± 7</td>
<td>78.2 ± 8</td>
<td>63 ± 2.6</td>
<td>68</td>
</tr>
</tbody>
</table>

Results are given as mean ± SD of 6 to 8 animals. For SPS, the calculation of the bioavailability was determined from extrapolated AUC after IV and subcutaneous injection of 250 anti-Xa U/kg.

Table 5. Duration of the Antithrombotic Effect (Wessler-Serum) After One Single Subcutaneous Injection (250 Anti-Xa U/kg) of CY216, NPS, or SPS

<table>
<thead>
<tr>
<th>Time After SC Injection (h)</th>
<th>Compound</th>
<th>Thrombus Prevention (%)</th>
<th>Plasma Activities (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anti-Xa</td>
<td>Anti-IIa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CY216</td>
<td>98 ± 1*</td>
<td>0.84 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>NPS</td>
<td>96 ± 3*</td>
<td>0.91 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>SPS</td>
<td>92 ± 4*</td>
<td>1.90 ± 0.06</td>
</tr>
<tr>
<td>12</td>
<td>CY216</td>
<td>22 ± 5</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>NPS</td>
<td>17 ± 6</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>SPS</td>
<td>68 ± 8*</td>
<td>1.10 ± 0.02</td>
</tr>
<tr>
<td>24</td>
<td>CY216</td>
<td>15 ± 4</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>NPS</td>
<td>20 ± 7</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>SPS</td>
<td>46 ± 9*</td>
<td>0.70 ± 0.03</td>
</tr>
<tr>
<td>48</td>
<td>SPS</td>
<td>38 ± 7*</td>
<td>0.25 ± 0.01</td>
</tr>
</tbody>
</table>

Results are given as mean ± SEM. Abbreviation: ND, not detectable.

* Significant antithrombotic effect (P < .01) in comparison with saline-treated animals.
reduction was far less than the 10-fold reduction observed for unfractionated heparin at similar doses. In contrast, clearances of NPS and SPS were independent of the dose delivered. But the most striking observation was the 10 times lower clearance of SPS by comparison with those of NPS and CY216. This difference may result from the very high affinity of SPS to ATIII, accounting for a half-life that tended to be close to that of the inhibitor. This explains the considerably higher plasma activities generated after continuous IV infusion or subcutaneous administration of the same dose of SPS in comparison with those of NPS and CY216. The slightly lower plasma activities recorded for NPS in comparison with those of CY216 suggest that, in the rabbit, the clearance of NPS is moderately higher than that of CY216, associated with a higher distribution volume (Table 2). The extrapolation of those pharmacokinetic parameters to the humans is not simple because there are large interspecies variations. By comparing the half-life of disappearance of NPS and of SPS in rat, rabbit, and baboon, and using an allometric model, the half-life of NPS has been predicted to be 18.2 hours in humans and that of SPS to be 96 hours. SPS was never delivered to human volunteers; however, in a recent phase I study, it was reported that the half-life of NPS ranged from 13 to 17 hours.

The duration of the antithrombotic effect after a single subcutaneous injection was evaluated in the stasis-Wessler model using human serum as thrombogenic stimulus. We have reported that this thrombogenic stimulus was more appropriate for studying the antithrombotic effect linked to the antifactor Xa activity of heparins than thromboplastin. As expected from the pharmacokinetic studies, high antifactor Xa activities persisted longer in the plasma for SPS than for NPS and CY216, and the antithrombotic effect was closely related to the circulating anti-Xa activity. These results also confirm several reports in the literature showing that pentasaccharide is an efficient antithrombotic agent in this stasis model.

The antithrombotic potencies of the three compounds were
then compared in a dynamic model of venous thrombosis growth. In this model, thrombin bound to preformed standardized thrombus converts plasma fibrinogen into fibrin and catalyzes its own formation by activating platelets, factor V, and factor VIII. The role of factor Xa bound to fibrin and platelets and its subsequent effect on prothrombin activation have also been recently emphasized.13,14 We have reported that, on the basis of equivalent antifactor Xa units injected, several LMWHs having different anti-Xa anti-IIa ratios were as potent antithrombotic agents as unfractionated heparin. However, LMWHs generate higher antifactor Xa activities than unfractionated heparin and various antifactor IIa levels. The present study provides the opportunity to make a direct comparison of the antithrombotic potency of an LMWH having an anti-Xa anti-IIa ratio of 3.8 with two compounds having a pure antifactor Xa activity. The curves describing the relationship between the doses, the subsequent plasma concentrations, and the resultant antithrombotic effect were not parallel. This finding suggests that different mechanisms are involved in the antithrombotic properties, namely an antithrombin and an anti-Xa effect for CY216, but a pure anti-Xa effect for the pentasaccharides. However, the data presented in Table 6 and Fig 2 indicate that pentasaccharides were almost as potent antithrombotic agents as CY216, provided that the plasma antifactor Xa activity was only 1.2 to 1.5 times higher. For SPS, these activities were obtained for total gravimetric doses 50 and 23 times lower than those required for CY216 and NPS, respectively.

Thus, at least in this experimental model, it is possible to inhibit venous thrombosis growth using pure antifactor Xa agents, compounds having both antithrombin and anti Xa effect such as unfractionated heparin13,14 or LMWHs, as well as specific antithrombin agents such as hirudin or dermatan sulfate.14,25 The best strategy among these different possibilities will be given by appropriate clinical trials. These in vivo observations contrast with the protective effect of fibrin on thrombin and factor Xa inhibition by the heparin or pentasaccharide-ATIII complexes that has been reported in vitro.3,25,26 They suggest that, during the amplification mechanisms triggered by thrombin bound to fibrin and ensuring venous thrombosis growth in flow conditions, free factor Xa is generated and that this enzyme reacts properly with pentasaccharide-ATIII complex.

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Pharmacokinetic and antithrombotic properties of two pentasaccharides with high affinity to antithrombin III in the rabbit: comparison with CY216

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