Isolation of a Heparin-Like Anticoagulant From the Plasma of a Patient With Metastatic Bladder Carcinoma

By Ayalew Tefferi, Barbara A. Owen, William L. Nichols, Thomas E. Witzig, and Whyte G. Owen

A 73-year-old woman with metastatic transitional cell carcinoma of the bladder developed vaginal bleeding a few days after undergoing radical cystectomy. She had no other signs of mucocutaneous bleeding. Coagulation studies revealed a markedly prolonged thrombin time (252 seconds), a slightly prolonged reptilase time (20 seconds), and mildly elevated fibrinogen (4.39 g/L), and fibrin D-dimer (200 to 500 ng/mL) levels. Treatment of the patient’s plasma in vitro with protamine or barium sulfate normalized the thrombin time. The anticoagulant activity corresponded to 0.15 heparin U/mL when measured by a thrombin time assay using normal plasma as substrate and standardized with porcine heparin. The anticoagulant was quantitatively bound to and subsequently eluted with 1 mol/L NaCl from quaternary aminoethyl (QAE) Sephadex, and then isolated by affinity chromatography on immobilized antithrombin III. The isolated anticoagulant was shown to be sensitive to heparinase digestion. Therefore, the inhibitor has functional and chemical properties similar to those of high-affinity heparin. Thus far, this is the only anticoagulant of this type isolated from the plasma of a patient bearing a tumor other than plasma cell myeloma.

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RESULTS

The results of the coagulation assays are summarized in Table 1. Notable was the marked increase in thrombin time, well out of proportion to the slightly increased reptilase time. The presence of an inhibitor was indicated by the finding that addition of an equal volume of normal plasma did not inhibit the patient’s plasma on the thrombin time assay. In the absence of the patient’s plasma, heparin (100 U/mL) decreased the thrombin time by 10 seconds, whereas addition of the patient’s plasma caused a 25-second prolongation. This anticoagulant was adsorbed with QAE Sephadex at 4°C for one hour. The anticoagulant was eluted from QAE Sephadex stepwise with increasing NaCl concentrations. The anticoagulant was isolated by affinity chromatography on a column of porcine antithrombin III noncovalently bound to concanavalin A-agarose. Polycrylamide gel electrophoresis of glycosaminoglycans was performed with a Phast system (Pharmacia AB, Uppsala, Sweden) using 8% to 25% gradient polycrylamide gels and native buffer strips. Gels were stained for glycosaminoglycan with Alcian Blue-Silver. The isolated anticoagulant was proteolytically digested with pronase (2 μg/mL) (Boehringer Mannheim Biochemicals, Indianapolis) or Heparinase (100 U/mL) (Seikagaku Kogyo Co, LTD, Tokyo) in 0.1 mol/L NaCl, 20 mmol/L Hepes, pH 7.0, overnight at 4°C in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

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correct the prolonged thrombin time. Plasma levels of factors V, VII, and X were within normal limits. The plasma factor VIII level was slightly elevated (52%, normal 83% to 117%). The thrombin time was normalized after the in vitro addition of either protamine or barium sulfate to the patient's plasma (Table 2). A protamine concentration in vitro of 100 μg/dL was required to completely correct the thrombin time, while a protamine concentration of 10 μg/dL corrected the thrombin time of normal plasma containing an equivalent amount (by anticoagulant activity) of porcine heparin (Table 2). The anticoagulant activity in the patient’s plasma corresponded to 0.15 heparin U/mL (Fig 1).

The anticoagulant was quantitatively bound to and subsequently eluted from QAE-Sephadex. All the anticoagulant activity in the patient's plasma corresponded to heparin-like substances. The resistance of the anticoagulant to neutralization by protamine is a feature ascribed to heparan sulfate in contrast to unfractionated or low molecular weight heparin. An unfractionated heparin of equivalent activity required approximately one tenth of the protamine sulfate needed to correct completely the patient's thrombin time (Table 2). However, the reduction in the anticoagulant activity obtained by heparinase digestion suggests properties of the anticoagulant more to resemble those of heparin. Upon electrophoresis of the patient's plasma, a band migrating slower than porcine heparin (Fig 2).

### Table 1. Coagulation Studies

<table>
<thead>
<tr>
<th>Coagulation Test</th>
<th>Patient (s)</th>
<th>Normal (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time</td>
<td>27</td>
<td>17-19</td>
</tr>
<tr>
<td>Partial thromboplastin time</td>
<td>115</td>
<td>25-40</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>&gt;600</td>
<td>21</td>
</tr>
<tr>
<td>Reptilase time</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>4.39 g/L</td>
<td>1.90-3.65 g/L</td>
</tr>
<tr>
<td>Fibrin split products</td>
<td>20 μg/mL</td>
<td>&lt;3 μg/mL</td>
</tr>
<tr>
<td>Fibrin D-dimer</td>
<td>200-500 ng/mL</td>
<td>&lt;200 ng/mL</td>
</tr>
<tr>
<td>Protamine gel test</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>32%</td>
<td>77% to 105%</td>
</tr>
</tbody>
</table>

### Table 2. Thrombin Times After In Vitro Addition of Protamine Sulfate

<table>
<thead>
<tr>
<th>Protamine Concentration (μg/mL)</th>
<th>Patient Plasma (s)</th>
<th>Heparinized Normal Plasma* (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>10</td>
<td>&gt;200</td>
<td>26</td>
</tr>
<tr>
<td>50</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>100</td>
<td>22</td>
<td>23</td>
</tr>
</tbody>
</table>

*Concentration = 0.15 units of porcine heparin per mL of normal plasma.

**DISCUSSION**

The prolonged thrombin time with a near-normal reptilase time in the patient's plasma is a characteristic effect of circulating heparin-like substances. The resistance of the anticoagulant to neutralization by protamine is a feature ascribed to heparan sulfate in contrast to unfractionated or low molecular weight heparin. An unfractionated heparin of equivalent activity required approximately one tenth of the protamine sulfate needed to correct completely the patient's thrombin time (Table 2). However, the reduction in the anticoagulant activity obtained by heparinase digestion suggests properties of the anticoagulant more to resemble those of heparin.
isolated anticoagulant containing five times the equivalent activity of the minimum detectable sample of porcine heparin, no material was detected by metachromatic or silver staining. Thus, either the anticoagulant expresses an exceptional specific activity or, more likely, is a conjugated proteoglycan that stains poorly. We have noted (unpublished observation, July 1988) that noncovalent heparin-antithrombin III complexes stain relatively weakly as compared with heparin or antithrombin III alone.

In the last 10 years at the Mayo Clinic, we have detected a total of five patients with circulating heparin-like anticoagulant activity. With the exception of the current report, all have been associated with plasma cell proliferative disorders: three with plasma cell myeloma and one with monoclonal gammopathy of undetermined significance (MGUS). All the patients manifested a thrombin time of >600 seconds that corrected in vitro by the addition of protamine and/or barium sulfate. Bleeding was severe in the patient with MGUS and in two of the patients with plasma cell myeloma. In one of the patients, high doses of continuous intravenous protamine sulfate (15 mg/h) were found to improve clinical bleeding and partially correct the in vitro clotting studies.

Although circulating heparin-like anticoagulant activity in humans has been associated mostly with plasma cell proliferative disorders, it can occur in other malignancies.

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