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 (>800 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.

© 1989 by Grune & Stratton, Inc.

The isolated anticoagulant was proteolytically digested with pronase (2 µg/mL) (Boehringer Mannheim Biochemicals, Indianapolis) or Heparinase (100 µ/mL) (Seikagaku Kogyo Co, LTD, Tokyo) in 0.1 mol/L NaCl, 20 mmol/L Hepes, pH 7.0, overnight at room temperature.

Case report. A 73-year-old woman presented with macroscopic hematuria in April 1987. Investigation revealed a high-grade transitional cell carcinoma of the bladder. The patient underwent radical cystectomy and ileal conduit urinary diversion. Regional and paraaortic lymph node biopsies showed metastatic involvement. Ten days after surgery, the patient developed significant vaginal bleeding, with a drop in her hemoglobin from 14.0 g/dL to 7.5 g/dL. There were no other signs of mucocutaneous bleeding. The intravaginal suture lines were intact and blood was diffusely oozing out of the intravaginal operative site. Coagulation studies suggested a circulating heparin-like anticoagulant (Table 1). The patient was not receiving heparin by any route of administration, including low-dose subcutaneous heparin or heparin flushes to keep intravenous lines open. Treatment of the patient’s plasma in vitro with protamine or barium sulfate normalized the thrombin time (Table 2). Infusion of intravenous protamine sulfate (20 mg) had no effect on either the vaginal bleeding or the in vitro clotting times. Higher doses of intravenous protamine sulfate were not tried because acceptable control of the vaginal bleeding was achieved through local measures and RBC transfusion requirements were limited to <1 U/d on the average. Although the patient’s in vitro clotting abnormalities persisted, she did not show any other signs of bleeding during her illness. Serum immunoelectrophoresis revealed no monoclonal protein. The patient died a few days later from lactic acidosis and renal failure; autopsy was refused.

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

© 1989 by Grune & Stratton, Inc.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

Submitted October 31, 1988; accepted March 22, 1989.

Supported by Grant HL-17430 from the National Heart, Lung, and Blood Institute, and by the Mayo Foundation.

Address reprint requests to Ayalew Tefferi, MD, Division of Hematology, Department of Internal Medicine, Mayo Clinic and Foundation, Rochester, MN 55905.

From the Division of Hematology, Section of Hematology Research, Department of Biochemistry and Molecular Biology, Mayo Clinic and Foundation, Rochester, MN.

© 1989 by Grune & Stratton, Inc.

0006-4971/89/7401-0075$3.00/0

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 while prothrombin was mildly decreased (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 \( \mu \)g/dL was required to completely correct the thrombin time, while a protamine concentration of 10 \( \mu \)g/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 0.15 heparin U/mL (Fig 1).

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. Upon electrophoresis of the patient’s plasma, the anticoagulant was not stained when native polyacrylamide gels and stained with Alcian Blue-Silver. The isolated anticoagulant was not stained when samples containing anticoagulant activity as high as 10 heparin equivalent U/mL were analyzed by this method.

The isolated anticoagulant was not stained when samples containing anticoagulant activity as high as 10 heparin equivalent U/mL were analyzed by this method.

**Table 1. Coagulation Studies**

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin time</td>
<td>27 s</td>
<td>17-19 s</td>
</tr>
<tr>
<td>Partial thromboplastin</td>
<td>115 s</td>
<td>25-40 s</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>&gt;600 s</td>
<td>21 s</td>
</tr>
<tr>
<td>Reptilase time</td>
<td>20 s</td>
<td>16 s</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 ( \mu )g/mL &lt;3 ( \mu )g/mL</td>
<td></td>
</tr>
<tr>
<td>Fibrin D-dimer</td>
<td>200-500 ( \mu )g/mL &lt;200 ng/mL</td>
<td></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 (( \mu )g/mL)</th>
<th>Patient Plasma (s)</th>
<th>Heparinized 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 gammapathy 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.

ACKNOWLEDGMENT

We thank Pam K. Fisher for her technical assistance.

REFERENCES


19. Griffith MJ, Marbet GA: Dermatan sulfate and heparin can be fractionated by affinity for heparin cofactor II. Biochem Biophys Res Commun 112:663, 1983


Table 3. Digestion of Anticoagulant With Hydrolases

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vol of Anticoagulant Added to 100 µL of Normal Plasma</th>
<th>Thrombin Time (s) (Control: 19 ± 1)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5.0 µL</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>2.0 µL</td>
<td>24</td>
</tr>
<tr>
<td>Pronase†</td>
<td>2.5 µL</td>
<td>24</td>
</tr>
<tr>
<td>Heparinase†</td>
<td>2.5 µL</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>2.5 µL</td>
<td>19</td>
</tr>
</tbody>
</table>

*Control thrombin times were repeated after each test; the range of values is indicated.

†Pronase or heparinase alone did not affect the control thrombin time values.
Isolation of a heparin-like anticoagulant from the plasma of a patient with metastatic bladder carcinoma

A Tefferi, BA Owen, WL Nichols, TE Witzig and WG Owen