Bovine thrombin-induced inhibitor of factor V and bleeding risk in postoperative neurosurgical patients

Report of three cases

JOEL A. SPERO, M.D.
Division of Hematology, Department of Medicine, Allegheny General Hospital, and Allegheny Campus, The Medical College of Pennsylvania, Pittsburgh, Pennsylvania

Three patients are reported who developed topical bovine thrombin-induced antibodies to clotting factor V following neurosurgical procedures. In each patient the coagulopathy occurred within 8 to 13 days following exposure to topical bovine thrombin at surgery. Two of the three had previously been exposed to bovine thrombin during cardiothoracic surgery. The three patients were identified by detection of a prolonged prothrombin time, ranging from 20.5 to 39.8 seconds. The patient with the highest factor V level (0.12 U/ml) experienced gastrointestinal bleeding, which ceased when the factor V level increased to more than 0.20 U/ml. One patient required a ventriculostomy. In that case the prothrombin time and factor V level fell to improve following administration of vitamin K, 10 units of fresh frozen plasma, and platelet transfusions; the factor V level temporarily increased from 0.03 to 0.32 U/ml following a 2-day course of intravenous gamma globulin (1 gm/kg/day). Plasmapheresis has also been reported to be of transient benefit in the treatment of this coagulopathy.

In most patients the factor V level rises and the prothrombin time improves toward normal within 3 to 6 weeks following surgical exposure. The individuals identified likely represent only a fraction of the patients who develop the coagulopathy. The latter either do not bleed or are not sufficiently challenged in the postoperative period for the bleeding risk to be tested. It is concluded that bovine thrombin-induced coagulopathy may occur following surgical exposure to topical bovine thrombin and may result in both postoperative morbidity and mortality in a subset of patients.

Key Words • bovine thrombin • factor V • plasma coagulopathy • coagulation inhibitor

This paper describes the occurrence of a postoperative coagulopathy not previously reported in neurosurgical patients. Prolonged thrombin clotting times following neurosurgery have recently been reported. Since topical bovine thrombin has been commonly used as a hemostatic agent during both neurosurgery and cardiothoracic surgery, it has been suggested that the observed prolonged thrombin clotting times resulted from exposure to topical thrombin at the time of surgery. The presence of an acquired inhibitor to clotting factor V in patients following cardiothoracic surgery has recently been reported.

Approximately 2000 neurosurgical and 2000 cardiothoracic surgical procedures are performed at Allegheny General Hospital each year. Three patients admitted to the neurosurgical service over a 2-year period were identified at the time of a coagulation consultation for determination of the etiology of a prolonged prothrombin time and activated partial thromboplastin time. The first patient (Case 1) was seen in July, 1990, the second (Case 2) in January, 1991, and the most recent (Case 3) in May, 1992. Two patients (Cases 1 and 3) had undergone prior cardiothoracic surgery: an aortic valve replacement in 1983 in Case 1 and coronary artery bypass grafting in 1977 in Case 3. Both patients were exposed to bovine thrombin at their prior surgery and again during their recent neurosurgery. Case 2 had no known prior definable exposure to bovine thrombin. During neurosurgery, the patients received either Thrombostat or Thrombinar as topical hemostatic agents.

Case Reports

Case 1

This 60-year-old man was admitted to the neurosurgical service in July, 1990, following a subarachnoid hemorrhage secondary to a ruptured cerebral aneurysm. He had been receiving warfarin, alternating 2.0 and 3.0 mg daily, because of a prosthetic aortic valve placed 7
years previously. His prothrombin time was maintained at 16 to 17 seconds. The warfarin was stopped and, three days after admission, when his prothrombin time was 11.9 seconds and his activated partial thromboplastin time was 32 seconds, he underwent surgery to resect the aneurysm. Prior to a scheduled cerebral angiogram 8 days later, when he had received no further warfarin, his prothrombin time was 20.7 seconds. He failed to respond to either parenteral vitamin K or a total of 10 units of fresh frozen plasma. The prothrombin time increased to 33.3 seconds and the activated partial thromboplastin time to 76 seconds. The factor V level was 0.04 U/ml and the factor VII level 0.82 U/ml. Thrombin clotting time was 14.4 seconds. Mixing studies showed correction of both prothrombin time and activated partial thromboplastin time toward but not reaching normal, and little improvement was seen in factor V levels following mixture with normal plasma. A diagnosis of an acquired inhibitor to factor V was made.

Two days later tests showed the following levels: factor II was 0.02 U/ml, factor V 0.03 U/ml, factor XI 0.12 U/ml, and factor XII 0.40 U/ml; the factor VIII, factor IX, and fibrinogen levels were normal. The patient proceeded to develop hydrocephalus, and a ventriculostomy was necessary. He received 10 platelet packs without improvement in the factor V level. Intravenous gamma globulin (1 gm/kg/day for 2 days) was administered and the factor V level increased from 0.03 to 0.32 U/ml on the day following the second dose. However, surgery was not attempted and the following day the factor V level fell to 0.11 U/ml, from which it slowly rose to 0.65 U/ml 4 weeks after the appearance of his coagulopathy. At that point the prothrombin time was 14.5 seconds and the activated partial thromboplastin time 38 seconds; 9 months later, these times were 13.4 and 32 seconds, respectively, and clipping of the remaining aneurysms was performed without hemostatic difficulty.

Case 2

This 79-year-old woman was admitted to the neurological service in January, 1991, following a subarachnoid hemorrhage. On admission, her prothrombin time was 12.2 seconds and her activated partial thromboplastin time 22 seconds. An aneurysm was identified by cerebral angiography, and right craniotomy with aneurysm clipping was performed 48 hours following admission. There was no history of prior cardiovascular surgery or neurosurgery.

The patient did well for 7 days, at which time she aspirated material and was transferred to the neurological intensive care unit. At 13 days postoperatively, her prothrombin time was 20.5 seconds and her activated partial thromboplastin time 47 seconds, and they failed to improve following administration of parenteral vitamin K. Her stool was guaiac-positive, and intermittent packed red blood cells were given. At 15 days postoperatively, the prothrombin time was 20.2 seconds and the thrombin clotting time 10.5 seconds; blood factor levels were as follows: factor V 0.12 U/ml, factor XI 0.16 U/ml, and all other factors normal. Mixing studies were similar to those performed in Case 1. Four days later, without treatment, the factor V level had risen to 0.28 U/ml and gastrointestinal bleeding had ceased. The patient was discharged without further difficulty.

Case 3

This 66-year-old man had undergone coronary artery bypass grafting in 1977. One year prior to his present admission, he experienced a deep venous thrombosis and a course of warfarin was begun. He was admitted to the neurosurgical service in May, 1992, with a large intracerebral hematoma. His prothrombin time was 22.9 seconds and his activated partial thromboplastin time 41 seconds. Following administration of fresh frozen plasma and vitamin K, his prothrombin time and activated partial thromboplastin time improved to 13.8 and 29 seconds, respectively, and the hematoma was evacuated. Except for fluctuations in behavior, the patient's condition slowly improved. Ten days after surgery, in anticipation of discharge, a prothrombin time and activated partial thromboplastin time were obtained and were 38.1 and 98 seconds, respectively. His thrombin clotting time was greater than 150 seconds; the factor V and factor XI levels were less than 0.06 U/ml. All other factor levels and the platelet count were normal. Mixing studies were performed as in Cases 1 and 2. The patient's prothrombin time peaked at 39.8 seconds 2 days later, then fell slowly. Four weeks following surgery, his prothrombin time was 22 seconds and his activated partial thromboplastin time 49 seconds; the factor V level had risen to 0.21 U/ml. He experienced no bleeding.

Laboratory Investigation

Prothrombin time (normal range 10 to 13 seconds), activated partial thromboplastin time (normal range 24 to 34 seconds), and thrombin clotting time (normal range 11 to 15 seconds) were obtained in these patients according to standard laboratory protocol. The plasma of the patient with a prolonged thrombin clotting time (Case 3) was treated with Heparin and all subsequent tests were performed on absorbed plasma. When no heparin was detected, testing was performed on native plasma. Clotting factor assays were carried out with commercial substrates in separate dilutions using one-stage test systems on a Coa-Screener.* Factors II, V, and VII were assayed with one brand of human substrate,† factors VIII, IX, X, and XII with another brand of human substrate, and factor XI with a bovine substrate.‡ The normal range of concentrations in humans for all clotting factors except fibrinogen is 0.50 to 1.50 U/ml. Clotting factor inhibitors were sought by repeating factor assays on a 50:50 mixture of patient plasma and normal plasma, incubated in a 37°C water bath for

---
* Coa-Screener manufactured by American Labor, Durham, North Carolina.
† Human substrate obtained from George King Bio-Medical, Inc., Overland Park, Kansas.
‡ Substrates obtained from Dade, Baxter-Travenol, Miami, Florida.
Postoperative bovine thrombin-induced inhibitor of factor V

1 hour, and testing for failure to normalize the clotting factor level of the mixture.

**Discussion**

Clinically unsuspected coagulopathies that may result in bleeding not only may confound a patient’s postoperative recovery, but also may lead to serious morbidity or mortality. Spontaneously acquired inhibitors to individual blood clotting factors in patients with previously normal levels are uncommon but, when they occur, may produce life-threatening hemorrhage. Acquired inhibition to clotting factor VIII has been the most commonly observed example of this category of coagulopathy and has generally been associated with a severe bleeding diathesis. Acquired inhibitors to clotting factor V are comparatively rare but, when observed, have caused a wide spectrum of hemorrhagic disease ranging from minimal to marked bleeding. Most commonly, the appearance of an anti-factor V antibody has been associated with the use of antibiotic agents or associated in time with the postoperative period following a variety of surgical procedures, including orthopedic, urological, gynecological, and general surgery.

In the past few years, several reports have described the appearance of antithrombin antibodies following cardiothoracic surgery and neurosurgery, manifested in the laboratory by prolonged coagulation screening tests: prothrombin time, activated partial thromboplastin time, and thrombin clotting time (Table 1). In the majority of cases, there has been no association with clinical bleeding. Zehnder and Leung in 1990 reported the case of a 65-year-old man who, at 7 to 10 days following cardiothoracic surgery, developed a heparin-induced associated with marked prolongation of his prothrombin time, activated partial thromboplastin time, and thrombin clotting time. He was treated with intravenous gamma globulin (0.8 gm/kg/day for 2 days), with no observed improvement in prothrombin time or activated partial thromboplastin time; he was then treated with prednisone and cyclophosphamide. On Day 23 postoperatively, he developed a deep-muscle hematoma and his chemotherapy was increased, epsilon-aminocaproic acid (Amicar) was given, and plasmaspheresis was initiated. An inhibitor to clotting factor V was first identified on postoperative Day 41. The deep-muscle hematoma and several subsequent bleeding episodes appeared to improve following plasmaspheresis. Rapaport, et al. recently reported two individuals who developed factor V inhibitors, one following cardiothoracic surgery and the second after orthopedic surgery during which a gelatin sponge soaked with topical bovine thrombin had been placed in the ileum site from which bone was removed for graft placement. An additional 20 patients, 11 from Pennsylvania and Indiana, six from the Mayo Clinic, and three from British Columbia, have been reported in abstract form.

**Pathophysiology**

The proposed mechanism of the development of the coagulopathy is that the patient is exposed to bovine factor V at surgery and it is recognized as an immunologically foreign antigen. The patient develops an antibody to the bovine factor V; the antibody then cross-reacts with the patient’s own human factor V, resulting in a deficiency of the protein and failure to respond to transfused factor V in the form of fresh frozen plasma. Bovine factor V has been demonstrated in the commercial topical thrombins with which patients are treated.

A consistent finding in the three neurosurgical patients reported here as well as in our cardiothoracic surgery patients was a very low factor XI activity level. The assay kinetics were those of an inhibitor (the assayed level rose with increasing dilution). The decrease in factor XI persisted much longer than the inhibition of either factor V or other clotting factors. It is possible that the profound effect on the factor XI assay system was related to the bovine substrate in which factor XI was assayed, because antibodies that developed to the bovine antigens in the topical thrombin may have interfered with the assay. Thrombin clotting times obtained with human thrombin were significantly shorter than those with bovine thrombin. This hypothesis concerning the impact of bovine reagents upon test results is presently being investigated. Less consistent was the finding of transient inhibitors of other clotting factors, such as factor II in Case 1. This assay was performed with human substrate. The clinical significance, if any, of the latter inhibitor remains unclear; however, the only consistent findings in all patients were inhibitors to factors V and XI.

It is not known why some patients develop antibodies and others do not. Since more than one-half of patients

<table>
<thead>
<tr>
<th>Authors &amp; Year</th>
<th>Case No.</th>
<th>Type of Surgery</th>
<th>TCT (sec)</th>
<th>PT (sec)</th>
<th>APTT (sec)</th>
<th>Factor V &gt;</th>
<th>Bleed (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diez-Martín, 1</td>
<td>neurosurgery</td>
<td>&gt; 600</td>
<td>-</td>
<td>NA</td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>et al., 1985 2</td>
<td>neurosurgery</td>
<td>&gt; 600</td>
<td>-</td>
<td>NA</td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stricker. 1</td>
<td>cardiothoracic</td>
<td>&gt; 120 33.5</td>
<td>73.5</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>et al., 1988 3</td>
<td>cardiothoracic</td>
<td>&gt; 120 15</td>
<td>44.5</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flaherty. 1</td>
<td>cardiothoracic</td>
<td>&gt; 300</td>
<td>-</td>
<td>NA</td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>et al., 1989 4</td>
<td>neurosurgery</td>
<td>&gt; 300</td>
<td>-</td>
<td>NA</td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>cardiothoracic</td>
<td>&gt; 300</td>
<td>-</td>
<td>NA</td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>cardiothoracic</td>
<td>&gt; 300</td>
<td>-</td>
<td>NA</td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zehnder &amp; Leung, 1990</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapaport. 1</td>
<td>orthopedic</td>
<td>&gt; 240 45.4</td>
<td>127</td>
<td>0.03</td>
<td>no</td>
<td></td>
<td></td>
</tr>
<tr>
<td>et al., 1992 2</td>
<td>cardiothoracic</td>
<td>&gt; 240 17.68</td>
<td>37</td>
<td>0.27</td>
<td>no</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* TCT = thrombin clotting time; PT = prothrombin time; APTT = activated partial thromboplastin time; = = level within normal limits; NA = value not available.
† Data obtained on postoperative Day 12.
‡ Data obtained on postoperative Day 41.
§ Data obtained on postoperative Day 40.
¶ Data obtained on postoperative Day 21.
in whom the coagulopathy develops do not bleed (also
for unknown reasons), it is a reasonable assumption
that many individuals develop subclinical anti-factor V
antibodies which are never discovered, since evaluation
of the prothrombin time and the activated partial
thromboplastin time is not routinely performed 8 to 12
days following surgery.

Therapeutic Considerations

Zehnder and Leung\(^{12}\) recommended plasmapheresis
as primary therapy for bleeding caused by bovine
thrombin-induced anti-factor V antibodies. However,
given the unpredictable benefit of apheresis in the treat-
ment of immunoglobulin G-mediated disease processes
and the success of intravenous gamma globulin in pa-
tients with spontaneously acquired inhibitors to factor
VIII,\(^{15}\) the latter treatment was utilized in our Case 1,
with at least a transient immediate response and correc-
tion of the defect over the subsequent couple of weeks.
Recovery may or may not have been hastened by intra-
venous gamma globulin therapy.

There is no way to predict which patients will bleed
subsequent to the development of an anti-factor V
antibody or its severity when bleeding does occur. We
have identified nine patients who developed a bovine
thrombin-induced anti-factor V antibody following car-
thoracic surgery, four of whom experienced bleeding;
the coagulopathy threatened a limb in one and likely
contributed to another patient's death.\(^1\) In this
group of nine patients, there was a suggestion that those
who bled had prolonged thrombin clotting times with
human thrombin as well as bovine thrombin, but this
remains conjecture.\(^1\) It also may be that the factor V
inhibitor appears earlier in patients with prior exposure
to bovine thrombin, such as in those who have under-
gone prior cardiothoracic surgery.

In individuals with anti-factor V antibodies second-
ary to bovine thrombin exposure who have bled, hem-
orrhagic risk and recovery appear to partially correlate
with the severity of the factor V deficiency and, when
present, bleeding has waned as the factor V level rises
above 0.15 to 0.20 U/ml. In general, a low factor V
level was present in all patients who bled; however, not
all patients with low factor V levels bled. In our patients,
the factor V level usually returned to normal within 3
to 6 weeks of bovine thrombin exposure.\(^{15}\) Interestingly,
the prothrombin time also returns toward normal
but may remain slightly prolonged for a year or more.
In patients with prosthetic mechanical cardiac valves,
the state of their anticoagulation is problematic. Most
of our patients have been maintained on a course of
aspirin.

Conclusions

In summary, neurosurgery patients who receive top-
ical bovine thrombin for hemostatic purposes are at
risk of developing a postoperative coagulopathy char-
acterized by the appearance of antibodies to clotting
factor V, with a resultant deficiency of factor V that
does not respond to blood product administration. As
many as 30\% to 40\% of such patients may bleed.

Some improvement in the factor V level following in-
travenous gamma globulin or plasmapheresis has been
observed. Neurosurgeons should use topical bovine
thrombin with discrimination and be aware of the
potential occurrence of this entity in exposed patients.

Acknowledgments

The author thanks Matthew R. Quigley, M.D., Richard E.
Prostko, M.D., and Donald W. Whiting, M.D., for the referral
of their patients for consultation; Brian L. Cmolik, M.D., for
aid in reviewing medical histories, Douglas A. Triplett, M.D.,
for helpful discussions, Ms. Carol Reid, for technical assist-
ance, and Ms. Pat Martin for preparation of the manuscript.

References

surgery: late bleeding complications from topical throb-
in induced factor V deficiency. J Thorac Cardiovasc
Surg 105:222–228, 1993
2. Diez-Martin J, Sikkink RA, Blackburn MN, et al: Ac-
quired inhibitor of bovine thrombin. Blood 66 (Suppl
1):320, 1985 (Abstract)
3. Diez-Martin J, Sikkink RA, Gilchrist GS, et al: Develop-
ment of anti-bovine thrombin antibodies following neu-
4. Feinstein DI: Acquired inhibitors of factor V. Thromb
Haemost 39:663–674, 1978
5. Flaherty MJ, Henderson R, Wener MH: Iatrogenic im-
munization with bovine thrombin: a mechanism for pro-
longed thrombin times after surgery. Ann Intern Med
111:631–634, 1989
cogulation factor V and thrombin associated with sur-
geal use of topical bovine thrombin or fibrin "glue." Blood
ificance of antibodies to bovine and human thrombin and
factor V after surgical use of bovine thrombin. Am J Clin
Pathol 97:84–91, 1992
clotting factor inhibitors primarily involving FV and FXI
occurring after cardiovascular and neurological proce-
9. Stricker RB, Lane PK, Leffert JD, et al: Development of
anti-thrombin antibodies following surgery in patients
10. Sultan Y, Kazatchkine MD, Nydegger U, et al: Intrave-
nous immunoglobulin in the treatment of spontaneously
acquired factor VIII: C inhibitors. Am J Med 91 (Suppl
11. Tsang PWK, Naiman SC: Development of antibodies
to bovine thrombin and to other coagulation factors in
(Abstract)
12. Zehnder JL, Leung LLK: Development of antibodies to
thrombin and factor V with recurrent bleeding in a pa-
tient exposed to topical bovine thrombin. Blood 76:

J. A. Spero

Manuscript received July 2, 1992.
Accepted in final form September 30, 1992.
Address reprint requests to: Joel A. Spero, M.D., Alle-
gheny General Hospital, 320 East North Avenue, Pittsburgh,
Pennsylvania 15212.