

Thrombosis Resulting from Heparin Therapy

Lisa L. Gottschling, Pharm.D., and Cesar Alaniz, Pharm.D.

Heparin is widely used in current practice for a variety of indications. It is well known that it can cause thrombocytopenia, but not that thrombosis may also develop in thrombocytopenic patients and cause significant morbidity and mortality. A 56-year-old woman developed heparin-induced thrombocytopenia with thrombosis that resulted in the amputation of her leg. It is proposed that the reaction has an immune-mediated mechanism. Several ways of diagnosing the condition are available, specifically the serotonin-release assay and an enzyme-linked immunosorbent assay. The investigational agent danaproid may be effective in the treatment of heparin-induced thrombocytopenia with thrombosis.

(*Pharmacotherapy* 1996;16(3):469–472)

Thrombocytopenia is a well-known adverse effect of heparin administration. Current estimates of frequency lie between 5% and 10% of patients who receive heparin.^{1–3} Thrombocytopenia itself may not present great danger, but these patients are at risk of developing a little-recognized and very dangerous adverse effect of heparin. Although it is rare (10–20% of those with thrombocytopenia),¹ platelet thrombi, or so-called white clots, may form in large arteries and veins of these patients. When untreated, significant morbidity and mortality can result, as high as 60% and 30%, respectively.^{4–7} A patient developed thrombotic complications while receiving heparin. She was eventually managed with the investigational agent danaproid (Orgaran, ORG-10172; Organon, West Orange, NJ).

Case Report

A 56-year-old woman was seen in the emergency department on transfer from her local hospital with a diagnosis of thrombocytopenia and disseminated intravascular coagulation (DIC). She was admitted to the local hospital 14

days earlier complaining of substernal chest pain with radiation to her left jaw, neck, and arm. Treatment began with intravenous heparin and nitroglycerin. She was eventually diagnosed as having had a subendocardial myocardial infarction, with a peak creatine kinase of 442 U/L.

One week after admission cardiac catheterization through the right femoral artery confirmed coronary artery disease. Shortly after this procedure the woman developed ischemia in her left leg secondary to emboli. She immediately underwent a left femoral embolectomy, left popliteal thromboembolectomy, and patch angioplasty of her left femoral artery.

Three days later an aortofemoral angiogram revealed acute thrombosis of the superficial femoral artery at the level of adduction. Urokinase thrombolytic therapy was attempted, but failed to relieve the ischemia, and gangrene began to develop. She underwent a left femoral-popliteal bypass with a saphenous vein graft. Despite these efforts, her left leg continued to deteriorate, and 12 days after admission the patient underwent an above-the-knee amputation.

During the course of these events the woman developed thrombocytopenia and signs of DIC despite the discontinuation of heparin. Progressive swelling of the right arm was noted for 1–2 days after surgery, and 14 days after her admission she was transferred to our hospital for further management.

On transfer she complained of excruciating left

From the Department of Pharmacy, William Beaumont Hospital, Royal Oak, Michigan (Dr. Gottschling), and the Department of Pharmacy, University of Michigan Medical Center, Ann Arbor, Michigan (Dr. Alaniz).

Address reprint requests to Cesar Alaniz, Pharm.D., Department of Pharmacy, University of Michigan Medical Center, 1500 East Medical Center Drive, Room B2D301, Box 0008, Ann Arbor, MI 48109-0008.

stump pain that required a patient-controlled morphine pump and around-the-clock meperidine. Her platelet count was reported from the other hospital as 36,000/ μ l. Heparin was strictly avoided and the woman was managed supportively.

Over the next 2 days her platelet count began to rise to 44,000/ μ l and 76,000/ μ l. Coagulation tests were performed (Table 1), and a method for anticoagulation was pursued. The diagnosis of heparin-induced thrombocytopenia with thrombosis (HITT) was made after a positive heparin-induced 14 C-serotonin platelet-release test. On the second day of hospitalization a Greenfield filter was placed and intravenous dextran 25 ml/hour was started.

Treatment with danaproid began 3 days after transfer on a compassionate use protocol (platelet count 73,000/ μ l). The patient was given a 2500-U bolus loading dose of danaproid followed by a continuous infusion of 400 U/hour, at which point intravenous dextran was discontinued. Warfarin therapy was instituted on day 7 and the patient's international normalized ratio was stabilized over 4 days. On hospital day 10 danaproid was discontinued and the woman was maintained with warfarin. Her platelets had completely recovered at this time and were 329,000/ μ l. The patient was discharged to rehabilitation on hospital day 21.

Discussion

Heparin-induced thrombocytopenia with thrombosis generally occurs 4–15 days after treatment is begun, although it may take less time if a patient has had exposure to the drug (1–9 days).^{1, 4, 5, 8, 9} The effect is not dose or route related (intravenous or subcutaneous),^{1, 4, 6, 10} and although some studies report a higher frequency with bovine heparin, it is thought that frequency is similar with both bovine and porcine sources of the drug.^{1, 4, 5}

The mechanism of HITT is not completely understood, but it is thought to be the result of a complex immunologic response.^{1, 3–6, 8, 11–14} This is in contrast to mild, transient thrombocytopenia due to heparin (>100,000 platelets), which is thought to be caused by a proaggregant effect of the drug on platelets, leading to sequestration by the spleen and reticuloendothelial system.^{3, 5, 12, 13} This reaction does not result in thrombosis and usually reverses even while drug administration continues.^{3, 13} In HITT, heparin binds to circulating platelet factor 4 (PF4) that has been released from activated

Table 1. Coagulation Values Measured During the First Days of Admission

Test	Result	Reference
Antithrombin III activity (mg/dl)	90	81–121
D-dimer (mg/ml)	0.5–1	0.0–0.25
Fibrinogen (mg/dl)	270	150–350
Fibrin split product (mg/ml)	32	< 8
Protein C activity (%)	103	62–126
Protein C antigen (%)	71	60–106
Protein S antigen (%)	62	43–132
Dilute Russell viper venom time ratio	1.1	0.1–1.1
14 C-serotonin release test (U/ml)	0.1	> 20% } positive
	100	< 20% }

platelets, and these macromolecular complexes are thought to be the major antigen.^{3, 11, 15} Antibodies directed against these complexes may then bind to platelet Fc receptors, resulting in fixation of complement that leads to platelet activation, release of α -granule contents (PF4, thromboxane B₂, serotonin, etc.) and aggregation, which repeats the cycle.^{3, 6, 8, 11, 12, 15, 16} It is also believed that endothelial-bound PF4 is an additional target for PF4-heparin antibodies, either IgG or IgM, that can lead to injury and a site for the formation of thrombosis.¹⁶

One study attempted to identify clinical or laboratory risk factors that may help predict which patients with heparin-induced thrombocytopenia are likely to develop thrombosis.¹⁷ Laboratory indexes studied included antithrombin III, heparin cofactor II, protein C, and factor X, which are all coagulation inhibitors. The authors reported similar decreases in all patients regardless of isolated thrombocytopenia or thrombocytopenia with thrombosis.

The indication for heparin treatment provided information for determining clinical risk factors in the study population. The risk of arterial thrombosis increased ($p < 0.001$) in patients receiving heparin for a cardiovascular indication (myocardial infarction, cardiac surgery). In addition, antecedent surgery ($p < 0.001$) appeared to be a risk factor for venous thrombosis. Malignancy, sepsis, and concurrent drug use did not correlate with thrombosis.¹⁷ Factors proposed by other authors as risks for development of thrombosis include atherosclerosis or vascular injury due to surgery, venous stasis, and increased numbers of platelet Fc receptors.¹²

Until recently, the diagnosis of HITT relied on clinical features and platelet aggregation assays. The latter are of limited value because of variability in methods, as well as low sensitivity

and specificity.^{3, 15, 18} Routine coagulation tests may help to distinguish HIT from other clotting disorders, but still cannot definitively diagnose the condition. For example, in contrast to DIC, fibrinogen levels and prothrombin time are usually normal in HIT, and fibrin split products are normal or slightly elevated.⁴

Currently, however, two assays are available that diagnose the reaction with greater specificity and attribute it to heparin use. The first test is performed by measuring heparin-induced platelet serotonin release. A control blood sample is obtained from a patient who is taking no drugs, and the platelets in this sample are labeled with ¹⁴C-serotonin. The serum from a heparin-treated, thrombocytopenic patient is added to the labeled platelet-rich serum and heparin is mixed into the vial in various concentrations. After a standard length of time the reaction is quenched, the sample is centrifuged, and the radioactivity of the supernatant is measured.²

The specificity of this test is based on the observation that patients who have a definite clinical diagnosis of HIT have a specific aggregation test result: at therapeutic concentrations, heparin induces platelet aggregation; at high concentrations, platelet aggregation is inhibited due to the fact that the high concentrations disrupt heparin-PF4 complexes and inhibit binding of the antibody.^{2, 11} Release of ¹⁴C-serotonin is a more specific marker of platelet activation than is a platelet-aggregation test.² A ¹⁴C-serotonin test is considered positive when more than 20% of total radioactivity is measured with 0.1 U/ml of heparin and less than 20% is measured with 100 U/ml of heparin.^{2, 17}

The immune nature of HIT means that it can also be detected by an enzyme-linked immunosorbent assay (ELISA). An ELISA is designed to detect the presence of antibodies directed against PF4-heparin complexes by coating a plate with the antigen itself. It appears from several studies that this is more specific and sensitive than ¹⁴C-serotonin release in detecting lower concentrations of antibodies.^{3, 16} Another possible advantage of the ELISA is that it can detect IgG, IgA, and IgM antibodies. The ¹⁴C-serotonin-release test can detect only IgG antibodies because it depends on platelet activation by specific Fc receptors (FcRII).¹⁵ A disadvantage is that it cannot detect cross-reactivity like the heparin-induced platelet activation (HIPA) test¹⁵ and it will not diagnose the condition if the reaction is the result of an antigen other than PF4-heparin complexes.^{3, 11}

The challenge with a patient who has developed HIT is how to continue anticoagulation if heparin must be discontinued. A number of different methods have been described in the literature, including the administration of aspirin, dipyridamole, low molecular weight heparins (LMWHs), intravenous dextran, and plasmapheresis. In our patient the investigational drug danaproid, a heparinoid, was obtained on a compassionate use basis. Danaproid is composed of low molecular weight glycosaminoglycans including heparan sulfate 84%, dermatan sulfate 12%, and chondroitin sulfate 4%. Its ratio of anti-Xa activity to anti-IIa activity is about 28 times greater than that of heparin¹⁸ and it has essentially no effect on platelets.¹⁹ This agent has even demonstrated inhibition of antibody-induced platelet aggregation and release of thromboxane B₂.^{20, 21}

A study of danaproid as an alternative anticoagulant in cases of HIT reported positive results.¹⁹ Of the 230 patients with heparin-induced thrombocytopenia, 159 also had at least one thrombotic complication. In 88 of these patients the complications were due to an immune reaction to heparin administration. The study showed that 194 (92.8%) of 209 patients were adequately treated with danaproid. Seven patients died probably or possibly due to the drug; causes were bleeding in one patient, thrombosis in five, and septic shock in one. Other bleeding events occurred in 40 patients, 14 serious, 26 minor.

In the same study, the cross-reactivity of danaproid with the heparin-induced antibody was compared with that of selected LMWHs (fraxiparine, fragmin, clexane [enoxaparin]). When a plasma sample tested positive for the heparin-induced antibody, an aggregation test was performed with danaproid and the LMWHs as well. Cross-reactivity for danaproid was less than 10% compared with greater than 90% for the LMWHs.¹⁹ Cross-reactivity also was addressed by a number of other studies using a variety of methods. Again, in each of them the cross-reactivity of LMWHs with heparin ranged from 78–94% and that of danaproid ranged from 10–18%.^{20, 21}

A higher concentration of the LMWHs than heparin is necessary to produce the reaction because it is at this higher concentration that optimum binding to PF4 takes place. The concentration appears to be inversely proportional to the molecular weight of the drug.³ Another factor that may affect cross-reactivity is

the drug's degree or grade of sulfation.²² One group studied the individual components of danaproid and found that dermatan sulfate and heparan sulfate have a low capacity for binding the PF4-heparin antibody.³

Clinical Application and Conclusion

Heparin-induced thrombocytopenia with thrombosis is not frequently encountered by practitioners, nor is it routinely considered in the differential diagnosis of spontaneous thrombi formation. Because it is very serious, however, medical staff must be alert to the possibility of a heparin reaction and monitor patients closely. A complete drug history from the patient is necessary. If an agent has caused HIT in the past, any form of rechallenge, regardless of length of treatment, can be fatal.¹⁹ Platelet counts should be measured regularly, because early detection of this disorder is critical; monitoring daily or every other day is recommended.^{1, 14} Appropriate action must be taken when a decreased platelet count is reported to prevent any deterioration in status.

Patients who develop thrombocytopenia should be examined for clinical signs of emboli such as pain and swelling in the extremities. If such symptoms are noted, heparin should be discontinued immediately and other anticoagulant therapy be instituted. A platelet aggregation or activation test should be performed to confirm the source of thrombocytopenia, preferably the heparin-induced serotonin-release test or PF4-heparin ELISA.

It has been suggested that an *in vitro* cross-reactivity test also be performed if the alternative agent is a glycosaminoglycan (enoxaparin or other LMWH, danaproid). If this result is negative, alternative therapy may continue while the clinical manifestations of thrombosis are monitored for improvement. Despite a negative cross-reactivity test result, an *in vivo* reaction cannot be ruled out because of low test sensitivity, especially in patients who are currently thrombocytopenic. If the test is positive, it is imperative that the patient be immediately converted to an agent that is not cross-reactive, or be given oral anticoagulant therapy, if possible.¹⁹ Although many recent advances have been made in the diagnosis and treatment of HIT, clinicians must continually be aware of the syndrome and its consequences.

References

1. Thomas D, Block JA. Thrombocytopenia, cutaneous necrosis and gangrene of the upper and lower extremities in a 35-year-old man. *Chest* 1992;102:1578-80.
2. Sheridan D, Carter C, Kelton JG. A diagnostic test for heparin-induced thrombocytopenia. *Blood* 1986;67:27-30.
3. Amiral J, Bridey F, Wolf M, et al. Antibodies to macromolecular platelet factor 4-heparin complexes in heparin-induced thrombocytopenia: a study of 44 cases. *Thromb Haemost* 1995;73:21-8.
4. Aburahma AF, Malik FS, Boland JP. Heparin-induced thrombocytopenia with thrombotic complications. *W Va Med J* 1992;88:95-100.
5. King DJ, Kelton JG. Heparin-associated thrombocytopenia. *Ann Intern Med* 1984;100:535-40.
6. Demasi R, Bode AP, Knupp C, Bogey W, Powell S. Heparin-induced thrombocytopenia. *Am Surg* 1994;60:26-9.
7. Heck HA, Lutchter CL, Falls DG. Fatal thrombocytopenic coagulopathy after cardiopulmonary bypass: clinicopathologic correlations implicating heparin. *South Med J* 1994;87:789-93.
8. Keeling DM, Richards EM, Baglin TP. Platelet aggregation in response to four low molecular weight heparins and the heparinoid ORG 10172 in patients with heparin-induced thrombocytopenia. *Br J Haematol* 1994;86:42-6.
9. Aster RH. Heparin-induced thrombocytopenia and thrombosis. *N Engl J Med* 1995;332:1374-6.
10. Hawkins KA, Schwartz MJ. Heparin-related thrombosis after myocardial infarction. *Am Heart J* 1984;108:1543-4.
11. Greinacher A, Pötzsch B, Amiral J, et al. Heparin-associated thrombocytopenia: isolation of the antibody and characterization of a multimolecular PF4-heparin complex as the major antigen. *Thromb Haemost* 1994;71:247-51.
12. Chong BH. Heparin-induced thrombocytopenia. *Br J Haematol* 1995;89:431-9.
13. Munver R, Schulman IC, Wolf DJ, Rosengart TK. Heparin-induced thrombocytopenia and thrombosis: presentation after cardiopulmonary bypass. *Ann Thorac Surg* 1994;58:1764-6.
14. Hirsch J, Dalen JE, Deykin D, Poller L. Heparin: mechanism of action, pharmacokinetics, dosing considerations, monitoring, efficacy, and safety. *Chest* 1992;102:3375-51.
15. Greinacher A, Amiral J, Dummel V, et al. Laboratory diagnosis of heparin-associated thrombocytopenia and comparison of platelet aggregation test, heparin-induced platelet activation test, and platelet factor 4/heparin enzyme-linked immunosorbent assay. *Transfusion* 1994;34:381-5.
16. Vinsentin GP, Ford SE, Scott JP, Aster RH. Antibodies from patients with heparin-induced thrombocytopenia/thrombosis are specific for platelet factor 4 complexed with heparin or bound to endothelial cells. *J Clin Invest* 1994;93:81-8.
17. Boshkov LK, Warkentin TE, Hayward CPM, Andrew M, Kelton JG. Heparin-induced thrombocytopenia and thrombosis: clinical and laboratory studies. *Br J Haematol* 1993;84:322-8.
18. Nicholson CD, Meuleman DG, Magnani HN, et al. Danaproid is not a low-molecular-weight heparin. *Am J Hosp Pharm* 1994;51:2049-50.
19. Magnani HN. Heparin-induced thrombocytopenia (HIT): an overview of 230 patients treated with Orgaran (Org 10172). *Thromb Haemost* 1993;70:554-61.
20. Cadroy Y, Amiral J, Raynaud H, et al. Evolution of antibodies anti-PF4/heparin in a patient with a history of heparin-induced thrombocytopenia reexposed to heparin. *Thromb Haemost* 1994;72:783-4.
21. Chong BH, Ismail F, Cade J, et al. Heparin-induced thrombocytopenia: studies with a new low molecular weight heparinoid, Org 10172. *Blood* 1989;73:1592-6.
22. Greinacher A, Feigl M, Mueller-Eckhardt C. Crossreactivity studies between sera of patients with heparin associated thrombocytopenia and a new low molecular weight heparin, reviparin. *Thromb Haemost* 1994;72:644-5.