Case Report

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Factor VIII inhibitors complicating treatment of postoperative bleeding in a dog with hemophilia A

Benjamin M. O'Kelley, DVM, Megan F. Whelan, DVM, DACVECC and Marjory B. Brooks, DVM, DACVIM

Abstract

Objective – To describe the clinical course of a dog with hemophilia A and circulating factor VIII inhibitors complicating the treatment of postoperative hemorrhage.

Case Summary – A 7-year-old castrated male Japanese Chin with hemophilia A, weighing 6 kg, was presented for dental cleaning, polishing, and extractions. Despite presurgical administration of cryoprecipitate, continuous oral bleeding occurred. Circulating factor VIII inhibitors were detected, and the postoperative hemorrhage was subsequently managed with extensive and prolonged blood component transfusion. The dog was discharged after a full clinical recovery.

New or Unique Information Provided – This case report describes the clinical consequences and successful treatment of postoperative hemorrhage in a dog with hemophilia A and circulating factor VIII inhibitors. A relevant discussion of the management of human patients with circulating factor VIII inhibitors is included.

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Introduction

Hemophilia A is a common X-linked recessive bleeding disorder caused by a specific deficiency of coagulation factor VIII (FVIII).[1,2] In canine and human patients, disease severity is classified based on residual FVIII coagulant activity (FVIII:C).[3,4] Human patients with severe hemophilia (FVIII:C < 2%) are at risk for frequent spontaneous hemorrhage and often require FVIII replacement therapy, whereas clinically milder forms are seen in patients with moderate (FVIII:C = 2–5%) or mild (FVIII:C = 6–20%) hemophilia.[1]

The development of inhibitory alloantibodies directed against FVIII represents the most severe complication of FVIII replacement therapy in humans. Such antibodies, known as circulating inhibitors, develop in approximately 20–35% of transfusion-dependent patients with severe hemophilia A.[5–7] Although less common, circulating FVIII inhibitors have been described in humans with mild to moderate hemophilia A.[5] Posttransfusion alloantibody formation is not unique to human patients. Inhibitory antibody formation has been described in a research colony of dogs with severe hemophilia A after transfusion of canine- (and human-) origin FVIII therapy.[3] Inhibitory alloantibody formation posttransfusion has also been described in a Labrador Retriever with severe hemophilia B (factor IX deficiency).[8] In the following report, we describe the clinical management of high-titer FVIII inhibitors in a dog with moderate hemophilia A.

From the Department of Emergency and Critical Care, Angell Animal Medical Center, Boston, MA 02130 (O'Kelley, Whelan) and the Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA (Brooks).

Address correspondence and reprint requests to
Dr. Benjamin M. O'Kelley, Department of Emergency and Critical Care, Angell Animal Medical Center, 350 South Huntington Avenue, Jamaica Plain, Boston, MA 02130, USA.
Email: bokelley@mspca.org

Case Summary

A 7-year-old castrated male Japanese Chin, weighing 6 kg, was diagnosed with moderate hemophilia A at a referral hospital when the dog experienced prolonged oral bleeding following a dental cleaning. Factor assays from a coagulation laboratory[a] revealed FVIII deficiency (FVIII:C = 4%; reference interval, 50–200%) and normal Factor IX coagulant activity (FIX:C = 57%; reference interval, 50–150%). The dog's oral bleeding was severe enough that his PCV dropped to 22%. He was treated with canine fresh frozen plasma (FFP) and packed red blood cell (PRBC) transfusions. Medical records indicate that the oral bleeding initially stopped

following a single 20 mL/kg FFP transfusion, but the dog had to be readmitted 72 hours later for recurrent bleeding and symptomatic anemia; PCV was 13% at that time. He was given PRBCs and approximately 40 mL/kg canine FFP; the oral bleeding stopped, with no rebleeding at that time.

Nearly 2 years later, the dog presented to a different referral hospital for a second dental evaluation and treatment of periodontal disease. The client's perspective was that the periodontal disease was severe enough that it adversely affected the dog's quality of life. Physical examination was normal with the exception of severe dental abnormalities. The prothrombin time was 6.7 seconds (reference, 7.3–11.8 s); the activated partial thromboplastin time (APTT) was 25.1 seconds (reference, <14.8 s). Platelet count and fibrin split products were within normal limits. The PCV was 51%; total protein (TP) was 82 g/L (8.2 g/dL). Blood type was dog erythrocyte antigen (DEA) 1.1 positive. Because of the dog's known history of hemophilia A and an anticipated need for dental surgery, 1 U (8 mL/kg) of canine cryoprecipitate obtained from a commercial blood bank[b] was administered.

An anesthetized oral examination revealed that gingivitis, gingival recession, dental calculus, and periodontal pocketing were present in varying degrees in all remaining teeth. Several teeth showed abnormal mobility. A total of 15 teeth were extracted; the remaining teeth were scaled and polished. Postoperatively, a cold compress was applied to the gingival incisions and an absorbable gelatin sponge[c] was packed into an area of the gums where slight bleeding had occurred intraoperatively. The dog's initial recovery from anesthesia was without complication.

The following day, minimal oral bleeding was noted; PCV initially dropped to 36%. Twelve hours later, the PCV was 38% and the dog was bright, alert, and responsive with a normal heart rate. APTT, measured on a point-of-care coagulation monitor,[d] was 110 seconds (reference, 87–97 s). The dog was discharged from the hospital, but developed oral bleeding at home 48 hours later, and was readmitted to the hospital. Mucosal sutures were observed to be intact, but a large soft blood clot was present surrounding the right upper canine, and blood was slowly but continuously oozing from this area of the gums. One unit of FFP (45 mL/kg), collected from a DEA 1.1 positive donor, was given over 4 hours. When the oral bleeding was still present the following morning, APTT was rechecked; it was severely prolonged at 128 seconds (reference, <14.8 s). PCV was 24%; TP was 58 g/L (5.8 g/dL). One unit of cryoprecipitate (11 mL/kg) was given over 3 hours.

The next morning, the APTT was 21.3 seconds, and PCV was 15%. One unit of cryoprecipitate (10 mL/kg) was given over 3 hours, and DEA 1.1 positive, cross-matched PRBCs (10 mL/kg) were administered over 3 hours. When oral bleeding continued, more FFP (20 mL/kg) and DEA 1.1 positive, cross-matched PRBCs (10 mL/kg) were administered. APTT, measured on a point-of-care monitor,[d] was 127 seconds (reference, <97 s). After another unit of cryoprecipitate (11 mL/kg) was given over 4 hours, the oral bleeding finally subsided. PCV was 33% and the dog's heart rate, mucous membrane color, and capillary refill time were all within normal limits. He was discharged from the hospital at that time.

Because of the unexpectedly high transfusion requirements and extended time interval required to stop the bleeding, the development of FVIII inhibitors was suspected. A citrated plasma sample (prepared from blood drawn into 1/10th volume of 3.8% sodium citrate) was shipped overnight on cold packs to a referral laboratory[a] for coagulation inhibitor testing. Factor VIII coagulant activity was measured in a modified 1-stage APTT assay, as described previously.[9] A screening test to detect FVIII inhibition[10] was performed by measuring the FVIII:C of an incubation mixture consisting of equal parts patient plasma and pooled canine control plasma (prepared from 20 healthy dogs). The results of these analyses revealed severe FVIII deficiency in the patient's baseline sample (FVIII:C = 1.5%) and a lack of detectable FVIII activity (FVIII:C<1%) in the patient/control plasma mixture. These findings were compatible with the presence of FVIII inhibitory activity in the patient plasma. The activity was quantified using the Bethesda assay method.[11] In brief, serial dilutions of the patient plasma were combined in a 1:1 ratio with pooled canine control plasma and incubated at 37°C. The residual FVIII:C of the incubation mixtures were assayed and compared with the FVIII:C of pooled control plasma combined 1:1 with saline. In this assay, the dilution of patient plasma containing residual FVIII:C of 50% represents 1 Bethesda Unit (BU) per milliliter of inhibitory activity. The Bethesda assay results revealed a high titer inhibitor of >256 BU/mL in the dog's plasma at the time of his discharge.

Two weeks later, a recheck physical examination indicated the oral surgery sites were healed; no oral bleeding was observed. The client reported that the dog was eating well at home and had regained a normal energy and activity level. PCV was 42%, TP was 70 g/L (7.0 g/dL). Citrated plasma was again submitted for FVIII:C and Bethesda inhibitory activity determinations. Assay results of the newly drawn sample revealed severe FVIII deficiency (FVIII:C = 1.5%) and persistent high-titer FVIII inhibitors (>256 BU/mL).

Discussion

Factor VIII is a nonenzymatic glycoprotein coagulation factor that circulates in plasma bound noncovalently to

von Willebrand factor (vWf).[12,13] This association with vWf both stabilizes the procoagulant activity of FVIII and prolongs its circulation by decreasing the rate of clearance of FVIII from the plasma and protecting it from proteolysis by factors IIa, Xa, and activated protein C.[7,12,13] Patients with hemophilia A have genetic mutations resulting in significantly decreased or absent FVIII:C. These mutations display genetic heterogeneity that translates into phenotypic heterogeneity. For example, humans with FVIII gene inversion produce no FVIII and are severely affected, while those with point mutations typically have quantitative or functional protein defects that result in relatively mild to moderate forms of hemophilia.[1] The variability in FVIII gene mutations has also been shown to influence the likelihood of circulating FVIII inhibitor development.[7]

While the exact mechanisms of alloimmunization in response to FVIII administration have not been fully defined, FVIII inhibitors develop most frequently in patients with severe hemophilia A.[5–7] Patients with severe hemophilia typically require repeated administration of exogenous FVIII to prevent or control recurrent hemorrhage. In most of these patients, the lack of functional FVIII results from inactivating mutations that abolish FVIII protein synthesis. In the absence of circulating endogenous FVIII antigen, transfused FVIII is presumably recognized by the patient's immune system as a foreign protein.[5–7] There also appears to be a genetic component of inhibitor development unrelated to the severity of hemophilia. A familial predisposition is supported by an observed increase in alloimmunization among first- and second-degree relatives of human patients with mild to moderate hemophilia who have developed FVIII inhibitors.[5]

The degree of hemostatic dysfunction resulting from the formation of FVIII alloantibodies depends on a number of factors, including epitope specificity and antibody titer.[6] Many inhibitory antibodies bind to functionally important domains of FVIII, interfering with its interaction with other coagulation factors or platelet phospholipids.[7] Other antibodies accelerate FVIII clearance (due to impaired vWF-binding) or enhance its proteolysis. Although clinically silent, many human hemophiliac patients develop non-neutralizing FVIII alloantibodies after transfusion.[6]

The medical management of FVIII inhibitors consists of short-term measures to control bleeding episodes and long-term approaches to modulate the immune response. Potential treatments for active hemorrhage include high-volume plasma-derived or recombinant FVIII concentrates, porcine FVIII, FVIII bypassing agents, antifibrinolytics, local hemostatic agents (such as absorbable gelatin sponges), and cold compresses. Human patients with posttransfusion antibody titers of $\leq$5 BU/mL are considered low responders and suitable candidates for high-volume FVIII administration strategies.[7,14,15] In these patients, administration of larger or more frequent amounts of exogenous FVIII may saturate existing antibodies, providing sufficient free FVIII for adequate hemostasis. The amino acid sequence of porcine FVIII is sufficiently different from the human protein to reduce its immunoreactivity, thereby prolonging the half-life of transfused porcine FVIII. Porcine FVIII is currently unavailable, however, due to contamination of plasma-derived products with endogenous porcine viruses.[16] Clinical trials are evaluating recombinant porcine FVIII as an alternative with no risk of pathogen transmission.

Acceptable sources of plasma-derived FVIII for the treatment of hemophilia A include FFP and cryoprecipitate. Cryoprecipitate is the treatment of choice because adverse reactions to cryoprecipitate appear to be less common than similar reactions to FFP.[2] The volume of FFP necessary to provide adequate exogenous FVIII may be very large, adding a risk for fluid overload. The patient of this report received both FFP and cryoprecipitate; optimally, only cryoprecipitate would have been used to decrease the risk of fluid overload.

Antifibrinolytic agents such as the lysine analogs epsilon-aminocaproic acid and tranexamic acid (TEA) bind plasminogen, preventing its activation to plasmin and resulting in reduced fibrinolysis. These agents can be administered systemically or applied locally, and can be especially useful following dental procedures. TEA mouthwash, for example, has proven to be as effective as FVIII replacement in controlling gingival hemorrhage in hemophiliac humans following dental scaling.[17]Although epsilon-aminocaproic acid and TEA have been used in dogs, their efficacy in controlling hemorrhage in canine hemophiliac patients with inhibitors remains unknown.

Some human patients with mild hemophilia develop FVIII inhibitors that react only with exogenous FVIII, sparing endogenous FVIII. Desmopressin (1–desamino-8-arginine vasopressin, DDAVP) may be effective in these patients by stimulating release of stored FVIII.[16] While the effects of DDAVP administration to dogs with FVIII inhibitors have not been described, administration of 0.4–5.0 μg/kg DDAVP subcutaneously to dogs with hemophilia A produced no substantial changes in FVIII activity in 1 study.[18]

If high-dose FVIII replacement is ineffective or not feasible, so-called bypass therapy may be used. Bypass therapy involves administering agents that allow for successful clot formation in the absence of properly functioning FVIII. The first available bypass agents were plasma-derived, activated prothrombin complex concentrates. More recently, recombinant activated

human factor VII (rhFVIIa) has proven effective as a bypass agent for controlling hemorrhage in human hemophiliacs with high-titer inhibitors.[15] Experimentally, infusion of rhFVIIa normalized the bleeding tendency of dogs with hemophilia A (measured by a secondary toenail bleeding time previously demonstrated to be abnormally prolonged in these patients).[19] Recombinant rhFVIIa administration was, however, highly immunogenic; it triggered an immediate urticarial response in 4 of the 5 dogs who received it, and all 5 dogs developed measureable antibodies against FVIIa.

An obvious concern with the use of bypass therapies is thromboembolic events caused by excessive procoagulant activity, but the incidence of such thrombotic events post-rhFVIIa administration in human hemophilia patients is low.[15] The risk of thromboembolic complications may be higher when bypass therapy and antifibrinolytic agents are used concurrently; this risk must be weighed when considering coadministration of these therapies to treat severe hemorrhage.[20]

Immunomodulatory approaches to the long-term management of coagulation inhibitors aim to eliminate antibodies using immunoadsorbtion or chemotherapy and immune tolerance induction (ITI) through sustained exposure to high-dose FVIII. ITI may be successful in eradicating inhibitors up to 80% of human patients with severe hemophilia A,[7] but the exceedingly high costs and limited availability of recombinant FVIII products prohibit ITI from being a realistic option for veterinary patients at this time.

While the development and clinical management of FVIII inhibitory antibodies is well characterized in the human medical literature, FVIII inhibitors have only been described in a single research colony of dogs with severe hemophilia A.[3] These dogs were all derived from 1 hemophilic Schnauzer sire, with inhibitor formation noted in the descendents of a single outcross female. Inhibitory antibodies in the affected dogs were classified as IgG, and cross-reacted with human and canine FVIII. When the affected dogs and their relatives were observed over a 10-year period, they demonstrated similar clinical features as human patients with inhibitors: transfusion resistance, heterogeneity of inhibitor expression between individuals and for a single individual over time, and initial response to porcine FVIII followed by development of anti-porcine cross-reactive antibodies.[21]

The development of FVIII inhibitory antibodies was suspected in the Japanese Chin reported here because of his failure to respond to appropriate transfusion therapy. Recommended FFP dosages for treating canine hemophiliac patients typically begin at 6–10 mL/kg, and recommended cryoprecipitate doses start at 1–2 mL/kg for such patients.[2,22] The volume of FFP needed to stop clinically relevant hemorrhage varies greatly among patients due to varying disease severity (FVIII:C levels) among patients and varying FVIII:C activity per unit volume of FFP.[2,22] In 1 study, FFP was administered to 4 dogs with mild to moderate hemophilia A.[2] The plasma was given at a volume of 1 U (250–300 mL)/15 kg body weight, and FVIII:C activity was tracked over time. Only 3 of the 4 dogs reached FVIII:C activities deemed likely to stop clinical hemorrhage based on previous studies correlating FVIII:C activity with cuticle bleeding time. The dog who did not reach an ideal FVIII:C was not screened for FVIII inhibitors, but the authors hypothesized that the presence of low-titer anti-FVIII antibodies could have been responsible for this dog's lack of response to FFP.

During the period of hospitalization described in this report, the Japanese Chin required a larger cumulative volume and more prolonged course of transfusion to control hemorrhage than after his initial dentistry. It is possible that re-exposure to canine FVIII induced an anamnestic response in this dog. The medical classification of high responder describes hemophilic patients that mount a robust immune response and rapid rise in FVIII inhibitor titer after FVIII replacement therapy. This patient's high-titer inhibitor (>256 BU/mL) posttransfusion is compatible with a high-responder classification.

The large volume of blood products (65 mL/kg of FFP and 40 mL/kg of cryoprecipitate) required to stop hemorrhage in this dog parallels reports in the human literature and highlights the difficulty of controlling active hemorrhage in high-responder hemophiliacs. Infusion of porcine FVIII or rhFVIIa was considered for this patient but the high cost, immunogenicity, and relative unavailability of these products lead to the decision to attempt high-volume transfusion. The goal of this approach was to administer enough exogenous FVIII to neutralize existing antibodies long enough to allow the patient's oral wounds to heal. The patient's small body size made it possible to give him large amounts of cryoprecipitate and FFP (on a mL/kg basis) without being cost prohibitive to his owners or depleting the hospital's supply of these blood products. Repeated application of a cold compress or local hemostatic agents (such as epinephrine or gelatin sponges) to the bleeding gums would have been useful for controlling hemorrhage in this patient, but sedation would have been needed because the patient was intolerant of these procedures. If bleeding continued despite these treatments, local or systemic antifibrinolytic administration may have been useful. Bypass therapy with rhFVIIa could have been considered if the bleeding became life threatening.

While apparently uncommon, canine hemophilia patients are at risk for immunologic transfusion reactions.

In addition to reports of coagulation inhibitors, alloantibodies directed against platelet antigens have been described in a German Shepherd with hemophilia A.[23] Hemophilic dogs that experience continuing hemorrhage despite cryoprecipitate or FFP transfusion should be screened to detect thrombocytopenia or FVIII inhibitors.

It is possible that the hemorrhage and subsequent need for PRBC transfusions could have been avoided in the dog of this report if the inhibitors had been detected by appropriate preoperative screening. Before elective surgery, dogs with known hemophilia and a history of exogenous FVIII administration should be tested to assess their present FVIII:C level and check for the presence of coagulation inhibitors. Clear endpoints to transfusion therapy should be met before any surgical procedure; normalization of the APTT or normalization of a modified toenail bleeding time should occur in response to adequate FVIII replacement. If clotting times do not normalize, or if inhibitors are detected before surgery, the necessity of the procedure should be carefully evaluated. Appropriate treatments, including antifibrinolytics, large volumes of cryoprecipitate, and bypass therapies should be available before proceeding with an elective procedure in these patients.

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Footnotes

[a] Comparative Coagulation Section, Animal Health Diagnostic Center, Cornell University, Ithaca, NY.
[b] Midwest Animal Blood Services, Stockbridge, MI.
[c] Gelfoam. Pfizer, New York, NY.
[d] SCA 2000. Synbiotics Corporation, San Diego, CA.

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