Case Series

Journal of Veterinary Emergency and Critical Care **25**(2) 2015, pp 273–277
doi: 10.1111/vec.12286

Use of enoxaparin in dogs with primary immune-mediated hemolytic anemia: 21 cases

Cynthia M. Panek, DVM; Reid K. Nakamura, DVM, DACVECC and Domenico Bianco, DVM, PhD, DACVIM

Abstract

Objective – To describe the complications and frequency of thrombosis associated with the use of enoxaparin, a low molecular weight heparin, in dogs with primary immune-mediated hemolytic anemia (IMHA).
Design – Retrospective case series.
Setting – Two privately owned veterinary referral hospitals.
Animals – Twenty-one client-owned dogs with primary IMHA.
Interventions – Dogs were treated with enoxaparin (0.8 mg/kg subcutaneously every 6 h) as the sole anticoagulation therapy starting at admission to the hospital.
Measurements and Main Results – Only 2 dogs had minor hemorrhagic complications associated with enoxaparin therapy. Frequency of thrombosis was not assessed. Long-term survival was comparable to other anticoagulation protocols reported for dogs with primary IMHA.
Conclusions – The use of enoxaparin was safe in a small group of dogs with primary IMHA. Whether enoxaparin therapy can reduce mortality and thrombotic complications in dogs with primary IMHA compared with other anticoagulation protocols remains unknown.

(J Vet Emerg Crit Care 2015; 25(2): 273–277) doi: 10.1111/vec.12286

Keywords: anticoagulant, heparin, IMHA, thrombosis

Abbreviations

aPTT	activated partial thromboplastin time
IMHA	immune-mediated hemolytic anemia
LMWH	low molecular weight heparin
UFH	unfractionated heparin

Introduction

Immune-mediated hemolytic anemia (IMHA) is the most common hemolytic disorder in dogs, and in the majority of cases is a primary or idiopathic disease.[1–4] The reported case fatality rate in dogs with IMHA ranges between 20% and 70%, and is highest during the first 2 weeks of therapy.[1–9] Venous thrombosis is a common complication of canine IMHA and is thought to account for up to 80% of the case fatality rate, primarily because the presence of thrombosis has been consistently documented at necropsy.[3–6] Dogs with IMHA have been reported to be hypercoagulable at hospital admission (ie, prior to initiating corticosteroid therapy)[9] and during hospitalization, based on analysis of plasma-based coagulation testing and whole blood thromboelastography.[8–11] Furthermore, IMHA is a common underlying disease in dogs that develop pulmonary thromboembolism and splenic and portal vein thrombosis.[12–14] Although the pathogenesis of thrombotic complications in animals with IMHA is not well understood, anticoagulant therapy has become part of the standard of care in dogs with IMHA.[15–17] Platelet activation is a consistent finding in dogs with IMHA, and ultra low dose aspirin or clopidogrel is commonly used for the prevention of thrombotic complications,[17–20] but the effectiveness of antiplatelet agents to prevent thrombosis remains unknown.[20] Individually adjusted dosing of unfractionated heparin (UFH) targeting specific plasma anti-Xa activity reduced case fatality in dogs with IMHA when compared with dogs with IMHA that received a fixed dose of UFH.[16] In a preliminary evaluation, the survival rate of dogs with IMHA that received individually adjusted dosing of UFH was also higher than a

From the Advanced Veterinary Care Center, Lawndale, CA (Panek, Nakamura); and Veterinary Specialists of the Valley, Woodland Hills, CA (Bianco).

The authors declare no conflict of interest.

Address correspondence and reprint requests to
Dr. Bianco, Advanced Critical Care, Emergency and Specialty Services, 20051 Ventura Boulevard, Woodland Hills, CA 91364, USA.
Email: dbianco@accessvetmed.com
Submitted January 10, 2013; Accepted November 29, 2014.

retrospective control group of dogs with IMHA treated with ultra low dose aspirin for thromboprophylaxis.[a] Because the individual response to a given dose of UFH in dogs is variable, the use of UFH in dogs with IMHA requires close monitoring using anti-Xa activity.[16,21] Measurement of activated partial thromboplastin time (aPTT) is another acceptable test used to monitor UFH therapy, but high-dose UFH has unpredictable effects on aPTT and resulted in hemorrhage in some dogs.[22]

In human medicine, low molecular weight heparins (LMWHs) are effective and safe anticoagulant medications, and may be superior to UFH for certain indications.[23–26] In contrast to UFH, LMWH is smaller and more homogenous in size, and is less likely to bind to plasma proteins, resulting in more predictable pharmacokinetics. LMWH, as used in human patients, does not require regular monitoring, and is a safer for at-home anticoagulation therapy.[23–27]

LMWHs have been studied in healthy dogs,[28,29] but data describing the use of LMWH in dogs with IMHA are limited.[22] In healthy dogs, enoxaparin[b] administered at a dosage of 0.8 mg/kg subcutaneously (SC) every 6 hours maintained target anti-Xa activity without hemorrhagic complications.[29] The purpose of this study is to retrospectively evaluate the safety of enoxaparin when used as the sole anticoagulant therapy in dogs with primary IMHA. We hypothesized that enoxaparin therapy at the dosage of 0.8 mg/kg SC every 6 hours would be safe in dogs with primary IMHA.

Materials and Methods

This retrospective study was conducted in client-owned dogs with newly diagnosed primary IMHA that were hospitalized at 2 veterinary referral hospitals located in Los Angeles County, CA, between January 2008 and January 2012. Inclusion criteria comprised dogs with primary IMHA that were treated with enoxaparin at a dosage of 0.8 mg/kg SC every 6 hours as the sole anticoagulant therapy, starting within 24 hours from admission to the hospital. Primary IMHA was diagnosed based on the presence of regenerative anemia (reticulocyte count greater than 60×10^9/L [60,000/μL]), evidence of hemolysis (eg, hyperbilirubinemia, bilirubinuria, hemoglobinemia, or hemoglobinuria), and at least 1 or more of the following criteria: (1) autoagglutination, (2) spherocytosis, (3) positive direct Coombs test. Dogs that had been exposed to any drugs, vaccines, or toxins less than 6 weeks prior to hospitalization, or were suffering from concurrent neoplasia or vector-borne disease, or were receiving other anticoagulant medications were excluded. Medical records were reviewed for presenting complaints, survival to discharge, survival at 6 months (based on recheck appointments or phone contact with pet owners), evidence of thrombosis (based on necropsy findings or clinical signs suspicious of thrombotic events, such as acute onset of neurologic, respiratory, or gastrointestinal distress, or unexplained ascites), major hemorrhage (defined as severe bleeding leading to hemodynamic compromise), minor hemorrhage (defined as mild bleeding not causing any need of blood product transfusion or prolonged hospitalization), duration of hospitalization, transfusion requirements, enoxaparin dosage, and duration of therapy.

Statistical analysis

Results are presented as median and range unless indicated otherwise. Descriptive statistical analyses were performed using a statistical software package.[c]

Results

Twenty-one dogs met the inclusion criteria. Presenting complaints included pale mucous membranes ($n = 16$), lethargy ($n = 14$), decreased appetite ($n = 7$), collapse ($n = 2$), vomiting ($n = 2$), and discolored urine ($n = 2$). Breeds represented included Cocker Spaniel ($n = 4$), mixed-breed dog ($n = 4$), Pitbull ($n = 2$), Labrador Retriever ($n = 2$), Shih Tzu ($n = 2$), and 1 each of the following breeds: Pomeranian, Maltese, Toy Poodle, Pug, Golden Retriever, Miniature Dachshund, and German Shepherd Dog. The dogs represented 13 spayed females, 7 neutered males, and 1 intact male. The age, body weight, rectal temperature, and initial clinicopathologic findings of the dogs included in the study are shown in Table 1. Results of a polymerase chain reaction (PCR) assay for vector-borne diseases (ie, *Anaplasma phagocytophylum*, *Anaplasma platys*, *Babesia canis*, *Babesia speciation*, *Bartonella henselae*, *Bartonella vinsonii*, *Ehrlichia canis*, *Ehrlichia* spp., *M. hemocanis/hematoparvum*, *Neorickettsia risticii*, *Rickettsia rickittsii*) were negative for all dogs. Results of thoracic radiography and abdominal ultrasonography were unremarkable in all dogs. All dogs were treated with oral prednisone (median dosage 2.2 mg/kg/d, range 1.6–3.4 mg/kg/d) or intravenous dexamethasone sodium phosphate (median dosage 0.31 mg/kg/d, range 0.24–0.45 mg/kg/d). Additional immunosuppressive medications were used in 17 dogs, starting on presentation in 5 dogs, and between 3 and 8 days following hospital admission in the other 12 dogs. The additional immunosuppressive therapies included azathioprine in 6 dogs, cyclosporine in 5 dogs, mycophenolate mofetil in 4 dogs, or leflunomide in 2 dogs. Other therapies included famotidine or omeprazole in 19 dogs, doxycycline in 16 dogs, and maropitant in 9 dogs. Following discharge from the hospital, corticosteroid doses

Table 1: Characteristics and clinicopathologic findings at hospital admission for 21 dogs with primary IMHA receiving enoxaparin therapy

Parameter	Study subjects ($n = 21$)
Age (y)	6.5 (1–13)
Body weight (kg)	15.1 (3.9–37.4)
Rectal temperature (°C; [°F])	38.3 [100.9] (37.4–39.5 [99.3–103.1])
Neutrophil count ($\times 10^9$/L; [$10^3/\mu$L])	23.4 [23.4] (5.4–46.3 [5.4–46.3])
Band neutrophil count ($\times 10^9$/L; [$10^3/\mu$L])	0.72 [0.72] (0.17–3.98 [0.17–3.98])
Hematocrit (%)	13.1 (9.5–21.2)
Platelet count ($\times 10^9$/L; [$\times 10^3/\mu$L])	132 (75–411)
Serum bilirubin (μmol/L; [mg/dL])	54.72 [3.2] (5.13–150.48 [0.3–8.8])
Serum albumin (g/L; [g/dL])	30.0 [3.0] (18.0–37.0 [1.8–3.7])
PT (s)	6.7 (6.0–8.3)
aPTT (s)	13.1 (10.5–14.6)

Data are expressed as median (range). PT, prothrombin time; aPTT, activated partial thromboplastin time.

were slowly tapered and then discontinued over a period of 6–15 months at the clinician's discretion.

During hospitalization, the median enoxaparin dose administered to patients was 0.81 mg/kg (range, 0.73–0.98 mg/kg), and the frequency of administration was every 6 hours in 20 dogs and every 8 hours in 1 dog. Enoxaparin therapy was discontinued at home in 17 of 18 surviving dogs following a gradually decreasing dosage over a range of 6–21 days (median, 15 d) and discontinued abruptly after discharge in the remaining dog. The enoxaparin dosage was decreased by maintaining the same dose but decreasing the frequency of administration to every 8 hours for the first 3–14 days following discharge, then every 12 hours for an additional 3–7 days, and then ceasing administration.

There were no identifiable immediate or delayed adverse drug reactions associated with enoxaparin administration. Major hemorrhagic complications were not noted in any dog during hospitalization or at home. All pet owners were contacted via phone and specifically asked about possible enoxaparin side effects. During at-home treatment with enoxaparin, 2 dogs experienced minor hemorrhagic complications (1 occurrence of injection site bleeding for each dog). All pet owners reported that the enoxaparin was easy to administer at home and that they were compliant about administering the enoxaparin.

Three dogs did not survive to discharge from the hospital, and necropsy was performed in 2. No secondary cause for IMHA was identified in either dog, but both had pulmonary venous thrombi. Over the 6-month follow-up period, 3 dogs relapsed with IMHA despite continued therapy with oral prednisone and were euthanized. Necropsy was performed in one of them and a mesenteric venous thrombus was identified. Clinical signs suggestive of thrombosis were not reported by any owners of dogs that survived the study period. The occurrence of thrombosis, duration of initial hospitalization, transfusion requirements, and incidence of relapse of the dogs in this study are shown in Table 2.

Table 2: Outcome measures of 21 dogs with primary IMHA receiving enoxaparin therapy

Outcome measures	Study subjects ($n = 21$)
Survival to discharge	18
Six-month survival	15
Hospitalization time (d)	4 (2–13)
Dose of pRBC transfusion (mL/kg)	19 (8–87)
Major hemorrhagic complications	0
Minor hemorrhagic complications	2
Suspicion or presence of thrombosis	3
Relapse of IMHA	3

Data are expressed as median (range). pRBC, packed red blood cell. Major hemorrhage is defined as severe bleeding leading to hemodynamic compromise, minor hemorrhage is defined as mild bleeding not causing any need for blood product transfusion or prolonged hospitalization.

Discussion

This retrospective case series demonstrated that enoxaparin given at a dose of 0.8 mg/kg SC every 8 hours was safe and well-tolerated in a group of dogs with IMHA. Only 2 dogs in this case series experienced minor hemorrhagic complications, and owner compliance for continued administration following discharge from the hospital was good.

Outcome evaluation in dogs with IMHA is difficult because of the wide variation in the severity of the disease, and comparisons among different published reports are complicated by diverse dog populations from different geographic locations, different immunosuppressive regimens, and the lack of a universally accepted scoring system. The survival of dogs receiving enoxaparin in this cohort is comparable with the results of that in other studies where high-dose UFH, individually

adjusted UFH, clopidogrel, or ultra low dose aspirin was used as thromboprophylactic agents, and is somewhat better than the survival in dogs with IMHA who received a fixed dose of UFH.[1,15–17,20] In some canine studies of experimentally induced thrombosis, enoxaparin in various dosing regimens and in combination with other anticoagulant drugs was superior to UFH in preventing thrombus formation,[30–34] but further studies are needed to determine the efficacy of enoxaparin therapy compared with other anticoagulant therapies in dogs with IMHA. Laboratory testing or diagnostic imaging that might have indicated the incidence of thrombotic events in surviving dogs was not evaluated in this study.

Monitoring of UFH and enoxaparin therapy in dogs has used anti-Xa activity target ranges that have been extrapolated from human medicine,[15,16,21,22,29] but the optimal target anti-Xa activity for enoxaparin therapy in dogs with IMHA has not been determined. The dose requirements for enoxaparin in dogs with IMHA may be higher than those in healthy dogs, as was seen when individually tailored UFH therapy was used for dogs with IMHA.[16] The relatively reliable pharmacokinetics of enoxaparin may make it a viable medication for anticoagulation in hospitalized dogs with IMHA. Because anti-Xa activity was not measured, meaningful conclusions regarding enoxaparin dosing in dogs with IMHA cannot be drawn from the present retrospective case series. Research studies in healthy dogs suggest that enoxaparin must be administered SC every 6 hours for effective inhibition of factor Xa activity.[28] Because of the administration frequency required for enoxaparin, and despite good owner compliance in this report, enoxaparin may be more feasible for use in hospitalized dogs rather than in those dogs being treated at home as part of chronic management following an acute IMHA crisis.

Limitations of this case series are inherent to its retrospective design, including the small sample size and a lack of randomization, control groups, masking, therapeutic monitoring, and standardized treatment protocol. Larger prospective, multicenter, randomized, placebo-controlled, double-masked, outcome-based studies using appropriate dosing and monitoring are needed to fully evaluate the efficacy and safety of enoxaparin therapy in dogs with primary IMHA. In conclusion, enoxaparin administered at the initial dose of 0.8 mg/kg SC every 6 hours appeared to be safe in a small group of dogs with primary IMHA.

Footnotes

[a] Orcutt ES, Polzin DJ, Armstrong PJ, et al. Comparison of individually monitored versus low-dose aspirin on survival of dogs with immune-mediated hemolytic anemia. (Abstr) J Vet Intern Med 2009; 23(3):693.

[b] Lovenox; Aventis Pharmaceuticals, Inc., Bridgewater, NJ.

[c] SPSS 14.0 for Windows, Microsoft, Redmond, WA.

References

1. Piek CJ, Junius G, Dekker A, et al. Idiopathic immune-mediated hemolytic anemia: treatment outcome and prognostic factors in 149 dogs. J Vet Intern Med 2008; 22(2):366–373.
2. Grundy SA, Barton C. Influence of drug treatment on survival of dogs with immune-mediated hemolytic anemia: 88 cases (1989–1999). J Am Vet Med Assoc 2001; 218(8):543–546.
3. McManus PM, Graig LE. Correlation between leukocytosis and necropsy findings in dogs with immune-mediated hemolytic anemia: 34 cases (1994–1999). J Am Vet Med Assoc 2001; 218(8):1308–1313.
4. Carr AP, Panciera DL, Kidd L. Prognostic factors for mortality and thromboembolism in canine immune-mediated hemolytic anemia: a retrospective study of 72 dogs. J Vet Intern Med 2002; 16(5):504–509.
5. Klein M, Dow S, Rosychuck R. Pulmonary thromboembolism associated with immune-mediated hemolytic anemia in dogs: ten cases (1981–1987). J Am Vet Med Assoc 1989; 195(2):246–250.
6. Reimer ME, Troy GC, Warnick LD. Immune-mediated hemolytic anemia: 70 cases (1988–1996). J Am Anim Hosp Assoc 1999; 35(5):384–391.
7. Swann JW, Skelly BJ. Evaluation of immunosuppressive regimes for immune-mediated hemolytic anemia: a retrospective study of 42 dogs. J Sm Anim Pract 2011; 52(7):353–358.
8. Goggs R, Wiinberg B, Kjelgaard-Hansen M, et al. Serial assessment of the coagulation status of dogs with immune-mediated hemolytic anemia using thromboelastography. Vet J 2012; 191(3):347–353.
9. Fenty RK, deLaforcade AM, Shaw SP, et al. Identification of hypercoagulability in dogs with primary immune-mediated hemolytic anemia. J Am Vet Med Assoc 2011; 238(4):463–467.
10. Scott-Moncrieff JC, Treadwell NG, McCullough SM, et al. Hemostatic abnormalities in dogs with primary immune-mediated hemolytic anemia. J Am Anim Hosp Assoc 2001; 37:220–227.
11. Sinnott VB, Otto CM. Use of thromboelastography in dogs with immune-mediated hemolytic anemia: 39 cases (2000–2008). J Vet Emerg Crit Care 2009; 19(5):484–488.
12. Johnson LR, Lappin MR, Baker DC. Pulmonary thromboembolism in 29 dogs: 1985–1995. J Vet Intern Med 1999; 13(4):338–345.
13. Laurenson MP, Hopper K. Herra MA, et al. Concurrent diseases and conditions in dogs with splenic vein thrombosis. J Vet Intern Med 2010; 24(6):1298–1304.
14. Van Winkle TJ, Bruce E. Thrombosis of the portal vein in eleven dogs. Vet Pathol 1993; 30:28–35.
15. Breuhl EL, Moore G, Brooks MB, et al. A prospective study of unfractionated heparin therapy in dogs with primary immune-mediated hemolytic anemia. J Am Anim Hosp Assoc 2009; 45:125–133.
16. Helmond SE, Polzin DJ, Armstrong PJ, et al. Treatment of immune-mediated hemolytic anemia with individually adjusted heparin dosing in dogs. J Vet Intern Med 2010; 24(3):597–605.
17. Weinkle TK, Center SA, Randolph JF, et al. Evaluation of prognostic factors, survival rates, and treatment protocols for immune-mediated hemolytic anemia in dogs: 151 cases (1993–2002). J Am Vet Med Assoc 2005; 226(11):1869–1880.
18. Weiss DJ, Brazell JL. Detection of activated platelets in dogs with primary immune-mediated hemolytic anemia. J Vet Intern Med 2006; 20(3):682–686.
19. Ridyard AE, Shaw DJ, Milne EM. Evaluation of platelet activation in canine immune-mediated hemolytic anaemia. J Small Anim Pract 2010; 51(6):296–304.
20. Mellett AM, Nakamura RK, Bianco D. A prospective study of clopidogrel therapy in dogs with primary immune-mediated hemolytic anemia. J Vet Intern Med 2011; 25(1):71–75.
21. Brooks MB. Evaluation of a chromogenic assay to measure the factor Xa inhibitory activity of unfractionated heparin in canine plasma. Vet Clin Pathol 2004; 33:208–214.
22. Scott KC, Hansen BD, DeFrancesco TC. Coagulation effects of low molecular weight heparin compared with heparin in dogs considered to be at risk for clinically significant venous thrombosis. J Vet Emerg Crit Care 2009; 19(1):74–80.

23. Iqbal Z, Cohen M. Enoxaparin: a pharmacologic and clinical review. Expert Opin Pharmacother 2011; 12(7):1157–1170.
24. Kadusevicius E, Kildonaviciute G, Varanaviciene B, et al. Low-molecular-weight-heparins: pharmacoeconomic decision modeling based on meta-analysis data. Int J Technol Assess Health Care 2010; 26(3):272–279.
25. Hirsh J, Rasch R. Heparin and low-molecular weight heparin: the seventh ACCP conference on antithrombotic and thrombolytic therapy. Chest 2004; 126:188S–203S.
26. Leizorovicz A, Cohen AT, Turpie AGG, et al. Randomized, placebo-controlled trial of dalteparin for the prevention of venous thromboembolism in acutely ill medical patients. Circulation 2004; 110:874–879.
27. Antman EM, Cohen M, McCabe C, et al. Enoxaparin is superior to unfractionated heparin for preventing clinical events at 1-year follow-up of TIMI 11B and ESSENCE. Eur Heart J 2002; 23(4):308–314.
28. Mischke R, Grebe S, Jacobs C, et al. Amidolytic heparin activity and values for several hemostatic variables after repeated subcutaneous administration of high doses of a low molecular weight heparin in healthy dogs. Am J Vet Res 2001; 62(4):595–598.
29. Lunsford KV, Mackin AJ, Langston VC, et al. Pharmacokinetics of subcutaneous low molecular weight heparin (enoxaparin) in dogs. J Am Anim Hosp Assoc 2009; 45:261–267.
30. Mestre M, Uzan A, Sedivy P, et al. Enoxaparin (Clexane, Lovenox), a low molecular weight heparin enhances t-PA-induced coronary thrombus lysis in anesthetized dogs without inducing hypocoagulability. Thromb Res 1992; 66:191–206.
31. Stassen JM, Rapold HJ, Vanlinthout I, et al. Comparative effects of enoxaparin and heparin on arterial and venous clot lysis with alteplase in dogs. Thromb Haemost 1993; 69:454–459.
32. Leadley RJ Jr, Kasiewski CJ, Bostwick JS, et al. Inhibition of repetitive thrombus formation in the stenosed canine coronary artery by enoxaparin, but not by unfractionated heparin. Arterioscler Thromb Vasc Biol 1998; 18:908–914.
33. Libersan D, Khalil A, Dagenais P, et al. The low molecular weight heparin, enoxaparin, limits infarct size at reperfusion in the dog. Cardiovasc Res 1998; 37:656–666.
34. Rebello SS, Kasiewski CJ, Bentley RG, et al. Superiority of enoxaparin over heparin in combination with a GPIIb/IIIa receptor antagonist during coronary thrombolysis in dogs. Thromb Res 2001; 102:261–271.

