Pharmacokinetics of Subcutaneous Low Molecular Weight Heparin (Enoxaparin) in Dogs

Unfractionated heparin has been the standard heparin used in human and veterinary medicine for its anticoagulation effect; however, it has a complex pharmacodynamic profile that requires close monitoring. Low molecular weight heparins have a more predictable bioavailability, allowing standardized dosing without individual patient monitoring. This project was designed to a) evaluate the pharmacokinetics of the subcutaneous (SC) administration of the low molecular weight heparin, enoxaparin, in dogs using anti-Xa activity as a marker of plasma enoxaparin concentrations and b) to establish the dose necessary to maintain activity within an established target range. Enoxaparin at 0.8 mg/kg SC *q* 6 hours consistently maintained target levels of anti-Xa activity in normal dogs without evidence of hemorrhagic complications. **J Am Anim Hosp Assoc 2009;45:261-267.**

Kari V. Lunsford, DVM, Diplomate ACVIM

Andrew J. Mackin, BSc, BVMS, MVS, DVSc, Diplomate ACVIM

V. Cory Langston, DVM, PhD, Diplomate ACVCP

Marjory Brooks, DVM, Diplomate ACVIM

From the Department of Clinical Sciences (Lunsford, Mackin, Langston), College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi 39762 and Comparative Coagulation Section (Brooks), Animal Health Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853-5786.

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Introduction

Enoxaparin[a] is one of several fractionated or low molecular weight heparin (LMWH) products derived through the depolymerization of naturally occurring porcine intestinal mucosal heparin.[1] The LMWHs were developed as an alternative to unfractionated heparin, the most commonly used form of heparin in both human and veterinary medicine. Unfractionated heparin has long been considered standard therapy in the prevention of venous and arterial thromboembolism in humans. It is widely used in veterinary medicine for animals with diseases associated with "hypercoagulability" and the development of thrombosis and pulmonary thromboembolism.[2-5]

Unfractionated heparin and LMWH inhibit clotting by accelerating the formation of irreversible complexes between the anticoagulant protein antithrombin (AT) and serine protease clotting factors IIa (thrombin) and Xa, and other serine protease factors involved in the coagulation cascade.[1,6] Heparin's interaction with AT is mediated by a unique pentasaccharide sequence; however, a longer chain length of 18 saccharides is required for the heparin-AT complex to bind with and inhibit thrombin. In contrast, formation of a ternary complex is not required for heparin's inhibition of factor Xa. Virtually all unfractionated heparin molecules contain >18 saccharide units, producing a ratio of anti-Xa/anti-IIa activity of 1:1.

Commercial LMWHs consist of shorter chain units that produce skewed anti-Xa/anti-IIa ratios, ranging from 2:1 to 4:1.[1,7-9] The shorter chain LMWH also demonstrates reduced binding to proteins and cells compared to unfractionated heparin, which in turn produces pharmacological properties proven beneficial for clinical use in human patients. The circulating half-life of enoxaparin following intravenous administration in humans is 4.4 hours, far longer than the half-life of unfractionated heparin (0.35 hours). As a result of this longer half-life, subcutaneous

(SC) enoxaparin is typically given to humans twice daily, whereas unfractionated heparin is usually given four times daily. Furthermore, the bioavailability (90%) of enoxaparin following SC injection in humans is far superior to that of standard heparin (29%) and is also far more predictable than unfractionated heparin.[10] The superior biological and pharmacological properties of enoxaparin compared to unfractionated heparin have made this LMWH a topic of much recent interest in the human literature and the subject of many ongoing experimental studies and clinical trials.[1,8,11]

Different LMWHs have somewhat different pharmacokinetic profiles in humans, and recent studies in animals illustrate important inter-species differences.[1,12,13] While a number of experimental studies evaluating enoxaparin have used dogs as models for human disease, such studies have tended to focus on intravenous drug therapy, and they are not relevant for optimizing SC enoxaparin therapy in this species.[11,14-17] To our knowledge, no pharmacokinetic studies of SC enoxaparin in dogs have been performed to develop a clinical dosage regimen. In the absence of canine-specific pharmacokinetic data, however, enoxaparin has been used empirically in clinical practice at a SC dose of 1 mg/kg *q* 12 hours. This dosage is extrapolated from human pharmacokinetic data.[18,19]

In our initial use of enoxaparin to treat dogs with immune-mediated hemolytic anemia at risk for pulmonary thromboembolism, we measured factor Xa inhibitory activity (anti-Xa activity) to assess the intensity of enoxaparin's anticoagulant effect (unpublished data). Data obtained from three clinical cases revealed that a dosing regimen of 1 mg/kg SC *q* 12 hours produced lower anti-Xa values than what were generally recommended for human thromboprophylaxis. A target therapeutic range of 0.5 to 1.0 U/mL anti-Xa activity is considered appropriate for human patients at risk for venous thromboembolism, whereas a somewhat higher range (0.5 to 1.8 U/mL) has been recommended for patients undergoing percutaneous coronary intervention.[1,20,21] While our studies found that two of the three dogs achieved anti-Xa activity within the range of 0.5 to 1 U/mL at the estimated time of peak drug activity (3 to 4 hours after drug administration), all dogs were well below that range at the 12-hour trough level (unpublished data).

These preliminary results suggested that the recommended SC enoxaparin dosage of 1 mg/kg *q* 12 hours for humans might not be directly applicable to dogs and that the pharmacokinetics of enoxaparin in dogs should be assessed. This study was undertaken to develop a dosage regimen for SC enoxaparin to produce a sustained anticoagulant effect in dogs, defined as an anti-Xa activity in the range of 0.5 to 2 U/mL.

Materials and Methods

The study subjects were seven healthy, young adult greyhounds that ranged in weight from 22.75 kg to 36.5 kg and were confirmed to be normal based on physical examination, complete blood cell counts, and serum biochemical analysis. Throughout all phases of the study, the dogs were examined daily for signs of spontaneous hemorrhage or other potential drug side effects. The study was approved by the Mississippi State University Institutional Animal Care and Use Committee.

Sample Handling and Laboratory Analyses

Samples for heparin monitoring and coagulation assays were drawn via jugular venipuncture. A 21-gauge Vacutainer needle was used to draw blood directly into 3-mL 3.8% sodium citrate tubes. The sample tubes were then centrifuged for 10 minutes at 1000 rpm, and the plasma was drawn off with plastic pipettes. The plasma was frozen (at least 1 mL per sample) at –40˚ to –70˚C in plastic sample tubes and was shipped on dry ice to Cornell University's Comparative Coagulation Laboratory.

At Cornell, a commercial kit[b] was used to measure the factor Xa inhibitory activity (anti-Xa) of enoxaparin. The assay is a one-step competitive inhibition assay, configured with a bovine factor Xa reagent and a chromogenic substrate of factor Xa, and it is used without the addition of exogenous AT as per manufacturer's instructions. The color change of the reaction mixture is inversely proportional to the enoxaparin concentration in the test plasma. The assay was performed using the manufacturer's standards and quality control materials.[c] In addition, a canine plasma control sample that was spiked with enoxaparin to contain 1.5 U/mL anti-Xa activity was assayed simultaneously with each run of test samples. The interassay coefficients of variation for the manufacturer's low- and high-concentration heparin controls and the spiked canine plasma control were 6.0%, 3.5%, and 9.0%, respectively.

Coagulation assays (activated partial thromboplastin time [aPTT] and prothrombin time [PT]) also were performed on each plasma sample using commercial reagents and an automated coagulation analyzer,[d] as previously described.[22]

Pharmacokinetic Study Phase One

Two healthy, adult dogs were each given a single injection of SC enoxaparin at a dose of 1.5 mg/kg. This starting dose was extrapolated from our findings (in our hospitalized animals) that a dose of 1 mg/kg did not sustain a desired anti-Xa activity (>0.5 U/mL; unpublished data). Blood samples were drawn at 0, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours following drug administration, and citrated plasma derived from these samples was shipped to the Comparative Coagulation Laboratory at Cornell University for analyses as described above. The resultant data were utilized to generate the biological effect over time curves, which were then used to calculate biological effect half-lives and optimum sampling times for Phase Two of the study.

Pharmacokinetic Study Phase Two

After a washout period of 2 weeks, the two dogs used in Phase One of the study were again administered increasing doses of enoxaparin to determine the relationship between the dose of enoxaparin given and its biological effect. Each

dog was given a single SC injection of enoxaparin at 0.7 mg/kg, 1 mg/kg, 1.5 mg/kg (data used from Phase One), and 2 mg/kg. Blood samples were again taken for analyses of anti-Xa activity at 0, 1, 2, 3, 4, 6, 8, 10, and 12 hours following drug injection. A washout period of at least 2 weeks was between each dose.

Dose-response curves were generated for the two dogs in Phases One and Two of our study, at enoxaparin doses of 0.7 mg/kg, 1 mg/kg, 1.5 mg/kg, and 2 mg/kg. Data were then used to calculate the optimum enoxaparin dose and dose interval required to maintain anti-Xa activity between 0.5 and 2 U/mL. This dose and dose interval were then evaluated in a larger group of dogs in Phase Three of the study. The PT and aPTT were measured in plasma drawn at the highest (2 mg/kg) enoxaparin dose prior to the administration of enoxaparin and at the time of expected peak anti-Xa activity 4 hours after dosing.

Pharmacokinetic Study Phase Three

Seven healthy, adult dogs (including the two dogs used in Phases One and Two of our study) were given SC enoxaparin at 0.8 mg/kg *q* 6 hours for nine doses. Blood samples for anti-Xa assay were drawn on day 1 at 0, 2, 3, 4, and 6 hours following drug injection. Blood samples were again drawn on day 3 at -3 (i.e., 3 hours prior to the ninth dose, which occurred on day 3), 0, 2, 3, 4, 6, 9, 12, 15, 18, and 24 hours following drug injection. The PT and aPTT were evaluated for each dog on day 1 prior to enoxaparin administration, and again on day 3, 3 hours after the last dose was administered.

Data Analysis

The pharmacokinetics of anti-Xa activity following enoxaparin administration were determined using standard pharmacokinetic approaches as provided in a commercially available software program.[e] As dose was in mg/kg while enoxaparin activity in plasma was reported in units of anti-Xa activity per mL, a conversion of 100 U/1000 μg of enoxaparin was used in order to convert plasma concentration to μg/mL and allow for calculation of the volume of distribution divided by the fraction absorbed (Vd/F). Parameters generated from a simultaneous fit of day 1 and steady-state concentrations included the elimination-rate constant and its associated half-life, total body clearance/F, and absorption-rate constant. The dosage regimen that was required to maintain an appropriate degree of anti-Xa activity was modeled based on these values.

Results

Phases One and Two

In the two dogs evaluated, peak anti-Xa activities of 1.41 and 1.56 U/mL were reached 4 and 3 hours, respectively, after a SC enoxaparin dose of 1.5 mg/kg, and the dog's 12-hour trough activities were 0.34 and 0.21 U/mL [Figure 1]. Both dogs attained peak anti-Xa activity 3 to 4 hours after enoxaparin administration at doses ranging from 0.7 to 2 mg/kg. At all but the highest dose (2 mg/kg), which had a trough level of 0.54 U/mL, the 12-hour trough level fell below 0.5 U/mL anti-Xa activity [Figure 2]. Baseline PT values fell within reference range (18 seconds for both dogs; reference range 13 to 18 seconds), as did aPTT values (13.5 and 12.5 seconds; reference range 10 to 17 seconds). At the highest dose (2 mg/kg) of enoxaparin administered, the peak PT value at 4 hours following drug administration was slightly prolonged (21.5 seconds) for one dog, and it remained unchanged from baseline (18 seconds) for the second dog. Peak aPTT values (16.5 and 17 seconds) remained within reference range for these dogs.

The dose response data from Phases One and Two were used to generate a pharmacological projection predicting

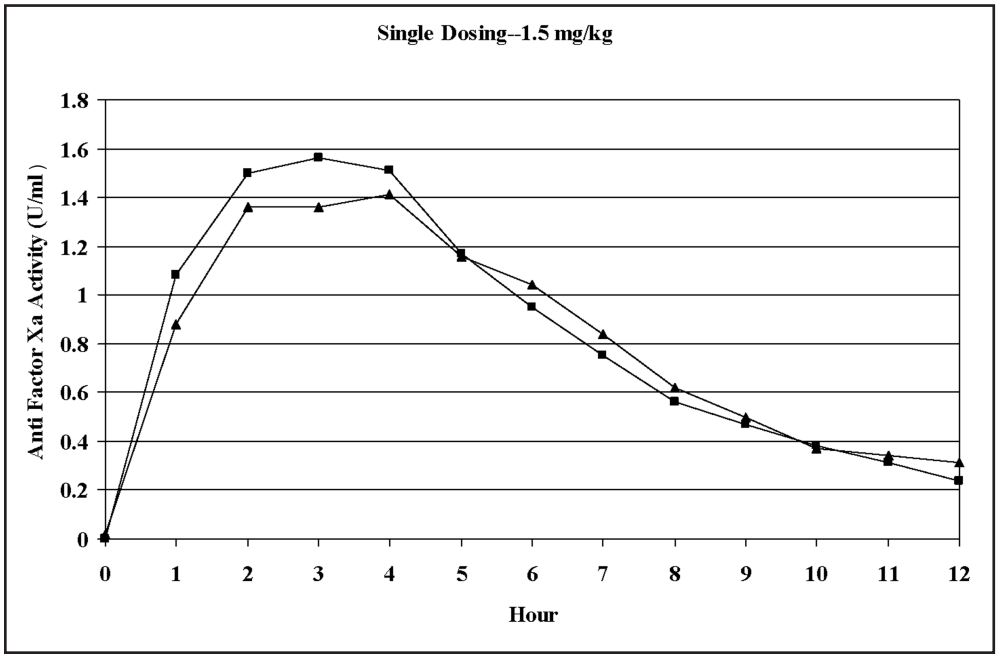

Figure 1—*Phase One*—Each dog reached a peak anti-Xa activity level well above the minimum target level of 0.5 U/mL, but both dropped below that level 9 hours following administration.

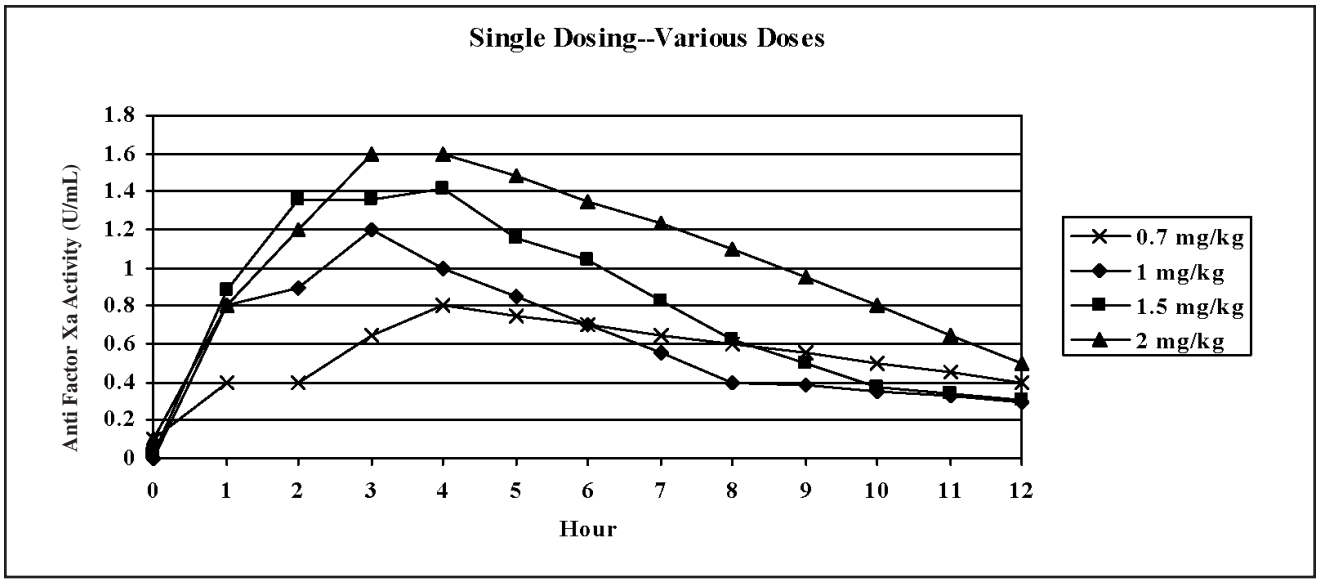

Figure 2*—Phase Two,* one dog—At all doses administered, peak anti-Xa activity occurred between 3 and 4 hours following drug administration. At all but the highest dose (2 mg/kg), the 12-hour trough anti-Xa activity level fell below 0.5 U/mL.

that a SC enoxaparin dose of 0.8 mg/kg administered *q* 6 hours would maintain therapeutic anti-Xa activity (between 0.5 and 2 U/mL) at steady state. Based on a calculated terminal half-life of approximately 5 hours, steady state was predicted to occur just over a full day of dosing (five half-lives).

Phase Three

Six hours after the first SC injection of enoxaparin, all but one of the dogs had achieved a peak anti-Xa activity level within the target therapeutic range [Figure 3]. The single dog that did not achieve anti-Xa activity by 6 hours did not have measurable Xa inhibition at any time interval during the first 6 hours after initial enoxaparin administration; therefore, this dog appeared to have missed receiving the initial drug dose. By day 3 (the first complete day after steady state was predicted to have occurred after receiving SC enoxaparin at 0.8 mg/kg *q* 6 hours), all dogs achieved both peak and trough anti-Xa activity levels within the target therapeutic range [Figure 4]. Anti-Xa activity then dropped to below target therapeutic levels (below 0.5 U/mL) between 6 and 9 hours after discontinuation of drug therapy for five of the dogs, but it remained within the therapeutic range at 15 hours for the other two dogs.

The disposition of enoxaparin was best described by a one-compartment, open extravascular model [see Table; Figure 5]. At a dose of 0.8 mg/kg SC, the mean (n=7) apparent specific Vd/F was 90 mL/kg, and the proximal and terminal half-lives were 0.69 hours and 5.10 hours, respectively.

The PT and aPTT were measured both before enoxaparin was administered on day 1 and at the expected peak anti-Xa activity 3 hours after drug administration on day 3, and no increase above the reference range was seen in any dog at any time. Peak anti-Xa activity PT values ranged from 11 to

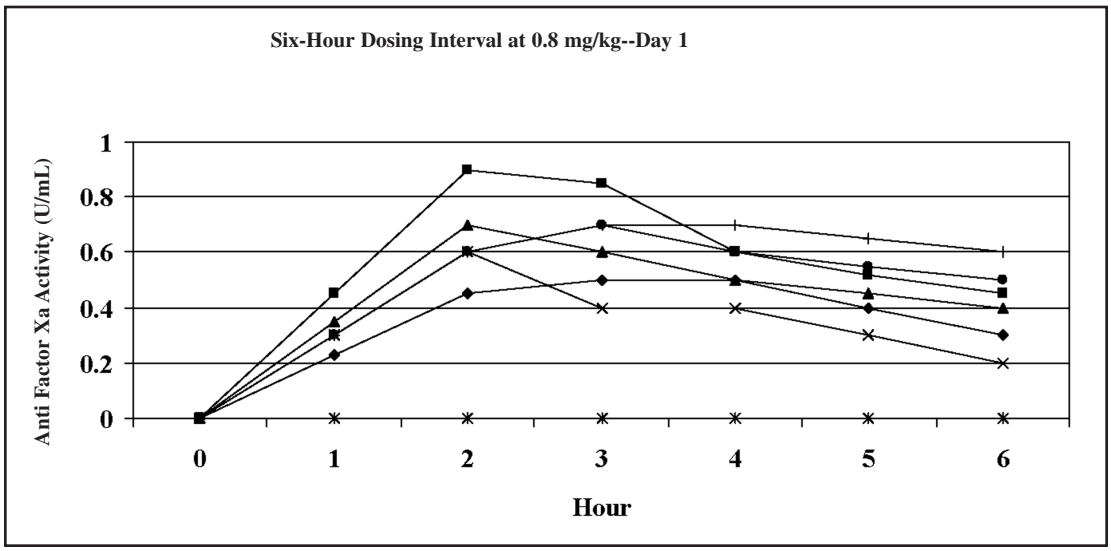

Figure 3*—Phase Three, Day One*—Six hours after the first subcutaneous infection of enoxaparin, all but one of the dogs had achieved a peak anti-Xa activity level within the target therapeutic range. Each line represents a single animal.

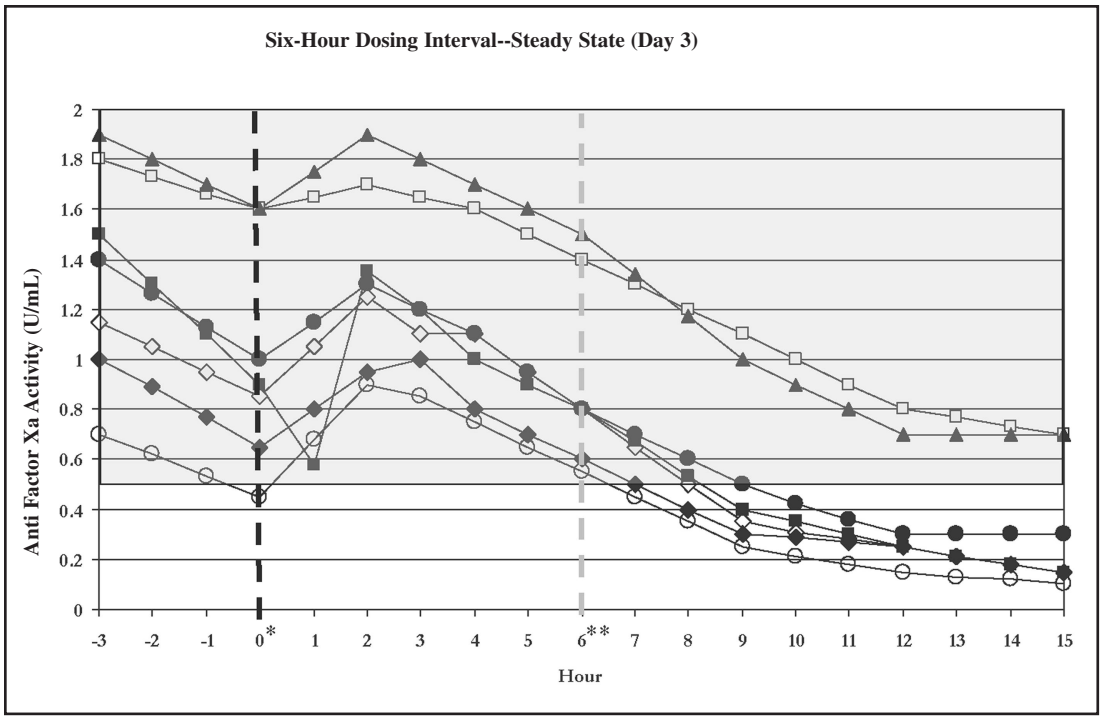

Figure 4*—Phase Three, Day Three*—All dogs achieved both peak and trough anti-Xa activity levels within the target range. Anti-Xa activity dropped to below target therapeutic levels between 6 and 9 hours for five of the dogs and remained above this level at 15 hours for two of the dogs.
* Last dose given.
** Next dose due.

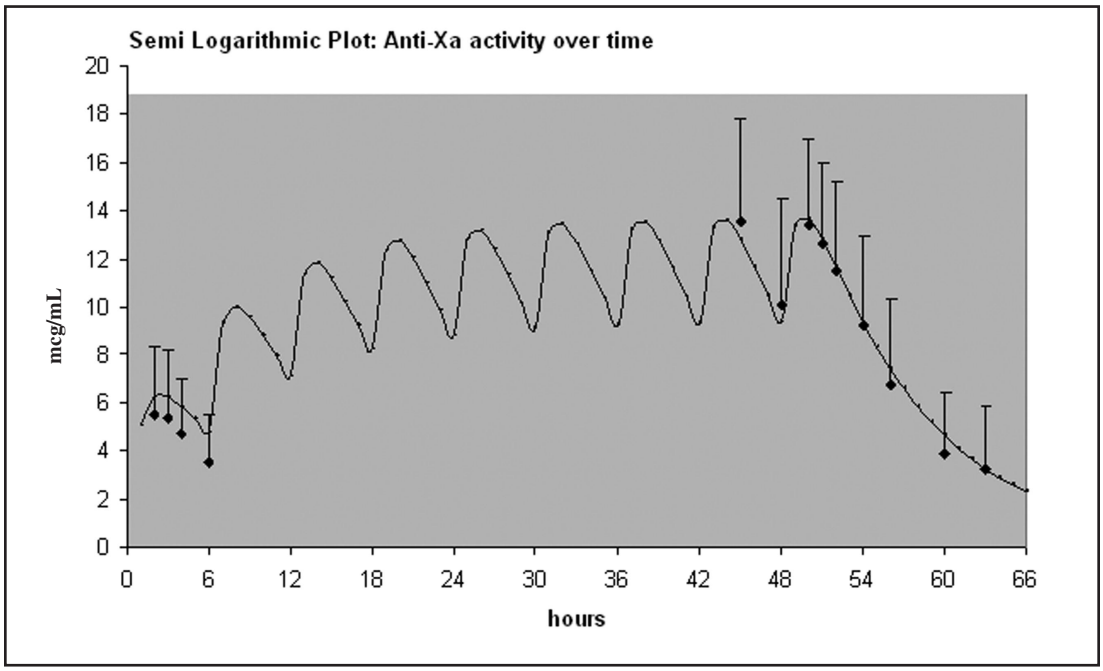

Figure 5*—*Semilogarithmic plot of mean (+ 1 standard deviation) plasma concentrations of enoxaparin activity following subcutaneous dosing of 0.8 mg/kg *q* 6 hours. Points were experimentally determined, and the line was calculated by use of a one-compartment extravascular model with the mean of the pharmacokinetic values.

16 seconds, and peak aPTT values ranged from 13 to 16.5 seconds. No signs of spontaneous hemorrhage or other potential drug side effects were detected in any dog at any time during the three phases of our study.

Discussion

Unfractionated heparin is a heterogenous mixture of molecules with a mean molecular weight of about 15,000 daltons (range 3000 to 30,000 daltons).[23] Heterogeneity in molecule size is associated with highly variable protein and cell binding and resultant inconsistencies in drug absorption, distribution, and clearance. Since a standard dose of unfractionated heparin does not have predictable effects, close monitoring of individual cases is essential. Doses must be carefully titrated, based on a target prolongation of aPTT or a target range anti-Xa activity, to minimize the risk of hemorrhage. Heparin molecules in LMWH formulations such as enoxaparin are, on average, much smaller than those in unfractionated heparin, with a mean molecular weight of only 4500 daltons (range 1000 to 10,000 daltons).[23] The superior pharmacokinetic profile of LMWH is largely due to the decreased binding affinity of smaller heparin molecules for proteins and cells. The LMWHs are replacing unfractionated heparins for many clinical indications in

Table

Mean Pharmacokinetic Parameters for Enoxaparin From a Simultaneous Fit of Day 1 and Day 3 Data Following Subcutaneous Dosing at 0.8 mg/kg *q* 6 Hours

Parameter*	Units	Mean	Standard Deviation
Vd/F	mL/kg	90.1	16.12
K01	/h	1.008	0.496
K10	/h	0.1357	0.0357
Cl/F	mL/h/kg	12.198	3.7947

Mean proximal half-life (h) 0.687†
Mean terminal half-life (h) 5.105†

* Vd/F=volume of distribution divided by fraction absorbed; K01 proximal rate constant; K10=terminal rate constant; Cl/F=total body clearance divided by fraction absorbed
† Half-life derived from mean rate constant

human medicine, because favorable pharmacokinetics allow standardization of a once- or twice-daily dosing regimen without frequent monitoring or dosage adjustments. The lower mean molecular weight of the LMWHs also reduces the proportion of heparin molecules that are large enough to bind to and inactivate factor IIa. Consequently, unlike unfractionated heparin, therapeutic dosages of LMWH do not prolong aPTT and activated clotting times. Therefore, guidelines for enoxaparin dosage in humans have been established using anti-Xa activity as a measure of anticoagulant intensity.[1,20]

In our pharmacokinetic study, anti-Xa activity was used to measure plasma enoxaparin concentration based on its biological activity. We found that SC administration of enoxaparin, throughout a dosage range of 0.7 to 2 mg/kg, consistently produced peak anti-Xa activity in healthy dogs at 3 to 4 hours following administration. Our study revealed that twice-daily enoxaparin dosing did not maintain anti-Xa activity within our predetermined target range; however, a dosing regimen of 0.8 mg/kg *q* 6 hours produced sustained anti-Xa activity within the desired range of 0.5 to 2 U/mL. This dosage regimen did not significantly prolong aPTT or cause any signs of abnormal hemorrhage, even in dogs with anti-Xa values at the high end of the target range (i.e., 2 U/mL). The target range of 0.5 to 2 U/mL was chosen by extrapolation from human studies. The relationship between target range anti-Xa activity and clinical outcomes, however, is somewhat controversial and still under investigation in clinical trials.[24,25] Some recent studies in the human literature have demonstrated improved outcomes in enoxaparin-treated patients who maintain anti-Xa activities within target range.[26,27] Pending clinical trials in dogs that relate specific anti-Xa activity levels with specific clinical endpoints, an enoxaparin dosage of 0.8 mg/kg *q* 6 hours is predicted to continuously sustain anti-Xa activity in the range of 0.5 to 2 U/mL, which is considered therapeutically relevant for humans.

In our study, a single enoxaparin dosage regimen maintained all treated dogs within target range at steady state, which demonstrated the predictability advantage of LMWH. This predictability reduces the need for continuous monitoring and "trial and error" dose titration associated with unfractionated heparin therapy. Enoxaparin has a longer half-life in humans than unfractionated heparin, thereby allowing for less frequent dosing. In contrast, in our study, the terminal half-life of enoxaparin in the dog was similar to that reported for unfractionated heparin.[28] Though a true volume of distribution (Vd) for enoxaparin cannot be calculated without an intravenous study, the Vd/F for SC enoxaparin of 90.1 mL/kg is consistent with values reported for the Vd of unfractionated heparin in dogs and humans where the drug is believed to be restricted to blood volume.[28] This may mean that the bioavailability of enoxaparin in the dog is high and absorption may approach 100%.

Based on our study in normal dogs, clinically ill dogs treated with enoxaparin should be given an initial SC dose of 0.8 mg/kg *q* 6 hours. Once steady state has been achieved (as early as 24 hours following the initial dose), a single measurement of "trough" anti-Xa activity should be measured 6 hours after the administration of a dose to confirm attainment of target drug levels. This starting enoxaparin dose has a high probability of rapidly attaining target anti-Xa activity, thereby eliminating a "subtherapeutic" window that often occurs during the first few days of dosage adjustment with unfractionated heparin therapy. The predictable and rapid effect associated with enoxaparin may prove particularly advantageous in dogs at high risk of systemic thrombosis and pulmonary thromboembolism. Greater anticoagulant predictability may also allow for safer at-home enoxaparin therapy, thereby reducing the time spent in the hospital. The reduced intensity of heparin monitoring and the possibility of earlier discharge from the hospital have compensated for the greater drug cost of enoxaparin in human practice, and this may prove applicable for management of thrombotic disease in dogs.

Since our study was performed in healthy dogs of a single breed (greyhound), our results cannot with certainty be directly extrapolated to other dog breeds or to dogs with various prothrombotic illnesses. Greyhounds are known to have hematological parameters (including increased packed cell volume and decreased platelet counts) that differ from those of other breeds, and recent work has shown that greyhounds may also have differences in metabolism and body composition sufficient to affect drug pharmacokinetics.[29,30] Further studies will be needed to confirm the pharmacokinetics of the biological effects of SC enoxaparin in relevant

disease and breed populations. Furthermore, clinical outcome trials are needed to test the hypothesis that enoxaparin dosage to attain the target anti-Xa range used in our study will improve survival for dogs with thrombosis and pulmonary thromboembolism.

Conclusion

We found that direct extrapolation of the standard human enoxaparin dose of 1 mg/kg SC *q* 12 hours was not sufficient to maintain plasma anti-Xa activity of healthy dogs within a target range. Enoxaparin given at a lower dose but more frequently (0.8 mg/kg SC *q* 6 hours) consistently maintained anti-Xa activity in the range of 0.5 to 2 U/mL. Enoxaparin appears to have a much more predictable biological effect than unfractionated heparin in dogs, and it may be preferable to unfractionated heparin for the prevention or treatment of thromboembolic disease.

Footnotes

[a] Lovenox; Aventis Pharmaceuticals, Inc., Bridgewater, NJ 08807
[b] Rotachrom Heparin; Diagnostica Stago, Parsippany, NJ 07054
[c] STA Calibrator HBPM/LMWH, STA Quality HBPM; Diagnostica Stago, Parsippany, NJ 07054
[d] STA Compact; Diagnostica Stago, Parsippany, NJ 07054
[e] WinNonLin; Pharsight Products, Mountain View, CA 94041

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