Pharmacodynamics of Multi-Dose Low Molecular Weight Heparin in Healthy Horses

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Abnormalities in coagulation in hospitalized horses are common and occur in up to 25% of septic foals,1 32% of horses with acute colitis,2 and 70% of horses with a large colon volvulus.3 Macroscopic thrombosis of the jugular vein is not uncommon in critically ill horses and thrombosis of other large blood vessels has been described occasionally.4–7 Histologic evidence of microthrombi formation and tissue fibrin deposition has been documented in 87% of necropsied septic foals and 47% of necropsied adult horses with inflammatory gastrointestinal disease.5,9 Derangements of coagulation in hospitalized horses are associated with longer hospitalization and increased mortality.1,3,7,10,11

Heparin has been administered to people and animals considered at risk for development of thrombotic complications and is the most commonly used anti-coagulant in horses.1,12,13 Currently, unfractionated heparin (UFH) and low molecular weight heparin (LMWH) formulations are available. UFH formulations contain chains of 18 or more saccharide units; they inhibit numerous activated clotting factors and interact with other plasma proteins making the degree and duration of their anti-coagulant effects difficult to predict.12,14–16 Erythrocyte agglutination, decreased platelet count, and hemorrhage have been associated with administration of UFH to horses limiting their use in the critically ill.16–20

LMWH formulations more specifically bind activated clotting factor X and interact minimally with other plasma proteins.21 In people, the increased specificity of LMWH has reduced the occurrence of side effects and results in a more predictable dose response allowing infrequent monitoring.21 These characteristics make LMWH formulations appealing as an anti-coagulant in equine patients; however, to date, only 2 pharmacodynamic17,22 and 3 clinical18,23,24 studies have reported the use of LMWH in horses. Once daily subcutaneous injection of an LMWH formulation (dalteparin; Fragnim, Pfizer, Inc., New York, NY) at 50 U/kg resulted in factor Xa inhibition in adult horses within or slightly above a range that has been suggested for thromboprophylaxis in people (0.1–0.2 U/mL) shortly after administration.17,22 However, factor Xa inhibition falls below this suggested range between 12 and 24 hours after administration and could be inadequate for up to 24 hours when given once daily. For both once and twice daily LMWH administration, the area under the curve was significantly greater after the last dose of LMWH when compared to the first dose.

Objectives: To compare the pharmacodynamics of once daily and twice daily administration of low molecular weight heparin (LMWH) administration in horses.

Study Design: Randomized cross over study.

Animals: Adult mixed breed healthy mares (n = 6).

Methods: LMWH (dalteparin) was administered (50 U/kg subcutaneously) either every 12 or 24 hours for 3 consecutive days. Anti-factor Xa activity was measured before and at select time points after LMWH administration. Packed cell volume (PCV), platelet count, partial thromboplastin time (PTT), and anti-thrombin (AT) activity were monitored throughout the study.

Results: No changes in PCV, platelet count, or AT activity were detected with either frequency of daily LMWH administration. Values for PTT increased throughout the study but never exceeded the normal reference interval. Anti-factor Xa activity was maintained within or above the suggested thromboprophylactic range (0.1–0.2 U/mL) when LMWH was administered twice daily, but fell below this range ~16 hours after administration when given once daily. For both once and twice daily LMWH administration, the area under the curve was significantly greater after the last dose of LMWH when compared to the first dose.

Conclusions: Administration of LMWH once or twice daily for 3 consecutive days appears to be safe in healthy adult horses. Anti-factor Xa activity was maintained within or above the suggested thromboprophylactic range for 24 hours with twice daily LMWH administration but not with once daily administration.
half the dosing interval if LMWH is administered only once daily.22 This is of concern because some human studies have suggested increased thrombotic complications when factor Xa inhibition is allowed to fall below the thromboprophylactic range.23

Our purpose was to determine the pharmacodynamics of LMWH administered at 50 U/kg subcutaneously every 12 hours to horses. We hypothesized that twice daily LMWH administration would maintain anti-factor Xa activity in horses within the suggested thromboprophylactic range more effectively than once daily administration.

MATERIALS AND METHODS

This study was approved by and performed according to the guidelines of the Institutional Animal Use and Care Committee. The experiment was a randomized cross over study enrolling 6 healthy mixed breed adult mares (age, 11–17 years; weight, 495–621 kg) from the veterinary teaching hospital’s research herd. Horses were determined to be healthy on the basis of a normal physical examination, complete blood count and plasma biochemistry analysis. Each horse was randomly assigned to initially receive either once or twice daily LMWH treatment for 3 consecutive days. After a washout period of at least 2 weeks, horses received the other treatment. Horses were housed in individual box stalls in a climate-controlled facility for the duration of LMWH administration and for 24 hours after the final dose. Free choice Coastal Bermuda hay and water were provided at all times. Before the study and during the washout periods, horses were kept at pasture. Physical examinations were performed every 12 hours during the study.

Medication Administration and Sampling Protocol

A 14 g 5½ inch intravenous (IV) catheter (BD Angiocath, BD Medical, Sandy, UT) was placed aseptically in one jugular vein at least 12 hours before the start of each study. LMWH (dalteparin; Fragmin, Pfizer, Inc.) was administered subcutaneously in the cervical region to horses at a dose of 50 U/kg (anti-factor Xa units) either every 24 hours (at 0, 24, and 48 hours) or every 12 hours (at 0, 12, 24, 36, 48, and 60 hours) for 3 consecutive days (Fig 1). Blood samples were collected from the IV catheter using a standard 3-syringe technique (6 mL blood was withdrawn and discarded, a 15 mL sample was collected and then the catheter flushed with 6 mL 50% dextrose). Samples were collected for measurement of anti-Xa activity and partial thromboplastin time (PTT) at the following times: before LMWH administration; every 12 hours; and at 1, 3, 6, 9, 12, 15, 24 (before the 1st dose) and then at 1, 3, 6, 9, 12, 15, 24 (before the 2nd dose), 36, 48 (before the last dose), 49, 51, 54, 57, 60, 63, and 72 hours (Fig 1). Sampling times were chosen to provide adequate time points for pharmacodynamic analysis; inclusion of the 3 hour time point was based on results of an earlier study in which anti-Xa activity peaked ~3 hours after LMWH administration.22

Packed cell volume (PCV) and total solids concentration were measured every 12 hours throughout the study. Anti-thrombin (AT) activity was measured before the first (time point 0) and last (i.e., at 48 or 60 hours) doses of LMWH because heparin requires adequate AT activity to function (Fig 1).12 Platelet numbers were also measured before the first and last LMWH doses as decreases in platelet counts have been reported following UHF administration to horses (Fig 1).17,22

Sample Processing

Measurement of Anti-Factor Xa Activity. A chromogenic assay measuring anti-factor Xa activity is considered the current standard for monitoring LMWH administration.21 Plasma was collected and stored at –80°C for batched analysis of anti-factor Xa activity (HemosIL, Instrumentation Laboratory, Bedford, MA) on an automated coagulation analyzer (ACL Elite, Beckman Coulter, Fullerton, CA). This assay has been validated for use in horses using equine plasma samples spiked with LMWH based on the methodology described by Brooks.26 For equine plasma samples with LMWH added to generate calculated anti-factor Xa activities of 0.2, 0.6, and 1.0 U/mL, intra-assay coefficient of variation is 4% and inter-assay coefficient of variation is 9.4%. The intra-assay coefficient of variation for this study was determined in 36 samples (6 samples from each of the 6 horses) in which anti-factor Xa activity was measured twice and ranged between 0.0% and 8.1%.

Measurement of PTT, AT Activity, and Platelet Counts. Stored plasma samples were also analyzed in batches for PTT on an automated coagulation analyzer (ACL 1000, Beckman
Coulter) and AT activity using a chromogenic assay (AMAX, Trinity Biotech PLC, Wicklow, Ireland). Platelet counts were determined from citrated whole blood samples immediately after collection on an automated hematology analyzer (CBC Diff, Heska Corporation, Loveland, CO).

**Pharmacodynamic Analysis**

For each horse, plasma anti-factor Xa activity versus time data were evaluated using noncompartmental analysis (PK Solutions 2.0, Summit Research Services, Montrose, CO). The elimination rate constant (Keq) was determined by linear regression of the terminal phase of the logarithmic activity versus time curve using a minimum of 3 data points. Terminal half-life ( t½) was calculated as the natural logarithm of 2 divided by Keq. The area under the activity-time curve (AUC0–∞) and the area under the first moment of the activity-time curve (AUMC0–∞) were calculated using the trapezoidal rule, with extrapolation to infinity using Cmin/Keq, where Cmin was the final measurable plasma anti-factor Xa activity. Mean residence time (MRT) was calculated as: AUMC/AUC. Apparent volume of distribution divided by bioavailability (Vdarea/F) was calculated as: dose/AUC/Keq and systemic clearance divided by bioavailability (CL/F) was calculated from: dose/AUC. For each horse, the time during which plasma anti-factor Xa activity were greater than 0.1 U/mL (T > 0.1 U/mL) was determined from the plasma activity versus time curve.

**Statistical Analysis**

Statistical analysis was performed with software (GraphPad Prism 5, GraphPad Software, La Jolla, CA). Normality of the data was assessed using the Kolmogorov-Smirnov test. Platelet counts were normally distributed and changes in platelet counts between the first and last dose were assessed using a paired Student’s t-test. AT activity was not normally distributed and changes in AT activity between the first and last dose were assessed using Wilcoxon-signed rank test. PCV and PTT were determined from citrated whole blood samples immediately after collection on an automated hematology analyzer (CBC Coulter) and AT activity using a chromogenic assay (AMAX, Heska Corporation, Loveland, CO). The elimination rate constant (Keq) was determined by linear regression of the terminal phase of the logarithmic activity versus time curve using a minimum of 3 data points. Terminal half-life ( t½) was calculated as the natural logarithm of 2 divided by Keq. The area under the activity-time curve (AUC0–∞) and the area under the first moment of the activity-time curve (AUMC0–∞) were calculated using the trapezoidal rule, with extrapolation to infinity using Cmin/Keq, where Cmin was the final measurable plasma anti-factor Xa activity. Mean residence time (MRT) was calculated as: AUMC/AUC. Apparent volume of distribution divided by bioavailability (Vdarea/F) was calculated as: dose/AUC/Keq and systemic clearance divided by bioavailability (CL/F) was calculated from: dose/AUC. For each horse, the time during which plasma anti-factor Xa activity were greater than 0.1 U/mL (T > 0.1 U/mL) was determined from the plasma activity versus time curve.

**RESULTS**

**Physical Examination and Clinicopathologic Findings**

Physical examination findings were normal throughout the study with the exception of small, focal, nonpainful subcutaneous swellings periodically noted at LMWH injection sites. Swellings were <5 cm diameter and resolved within 48 hours of drug administration. Platelet counts and AT activity before the first and last LMWH doses were not significantly different for either treatment frequency. No statistically significant changes in PCV were detected during the study with either once or twice daily LMWH administration. For once daily LMWH administration, a significant temporal change in PTT was detected (P = .003); however multiple pairwise comparisons did not detect any significant differences between specific time points. For twice daily administration, a significant temporal change in PTT was also detected (P < .0001); however, multiple pairwise comparisons did not detect any difference from baseline values. Values for PTT never exceeded the normal reference interval with either once or twice daily LMWH administration. The highest value for PTT was recorded in the twice daily administration group 6 hours after the last dose of LMWH (median, 65.9 seconds; range, 62.4–78.2 seconds; normal reference interval, 47.8–82.5 seconds).

**Anti-Factor Xa Activity**

Figure 2 shows mean plasma anti-factor Xa activity for the 24 hours after the first and last doses of LMWH (50 U/kg) administered subcutaneously to healthy horses either once or twice daily. Repeated analysis of plasma samples from horse 4 did not show any increase in anti-factor Xa activity above baseline at 3 and 6 hours after administration of the 1st LMWH dose in the twice daily treatment study. Anti-factor Xa activity in this horse increased appropriately after the 2nd (12 hour) and all subsequent LMWH doses and at all time points during the once daily treatment study. Consequently, values for anti-factor Xa activity from this horse for the first 12 hours of the twice daily LMWH administration study were excluded from analysis.

**Pharmacodynamics**

Pharmacodynamic variables comparing once and twice daily LMWH administration are shown (Table 1). There were no statistically significant differences in the maximum anti-Xa activity (Cmax), time to maximum anti-Xa activity (Tmax), t1/2, MRT, and Vdarea/F for frequency of administration (i.e., once versus twice daily LMWH administration) or for the first versus last dose of LMWH (Table 1). There was no statistically significant difference in the AUC for frequency of administration; however, the AUC was significantly greater after the final dose when compared to the 1st dose for both once and twice daily LMWH administration (Table 1). Similarly, clearance decreased significantly between the first and last doses but the frequency of administration had no significant effect (Table 1). For both once and twice daily LMWH administration, the AUC0–∞ after administration of the 1st dose was not significantly different (P = .765 and P = .319 for once daily and twice daily administration, respectively) from the AUC0–∞ after administration of the last dose, indicating that steady-state anti-Xa activity had been reached. At steady state, the time for
which anti-factor Xa activity exceeded 0.1 U/mL was significantly longer when administered twice daily (24.0 ± 0.0 hours) compared to once daily administration (15.9 ± 2.8 hours; Table 1).

**DISCUSSION**

As in previous studies, subcutaneous administration of the LMWH formulation dalteparin at 50 U/kg was well tolerated by healthy adult horses with no clinically relevant adverse effects.\(^{17,18}\) Further, the decreases in PCV and platelet count often associated with UFH therapy were not observed in horses administered dalteparin either once or twice daily for 3 days.\(^{17,18}\) As previously reported for people and horses, values for PTT remained within the normal reference interval after dalteparin administration despite an increase in factor Xa inhibition.\(^{17,21}\) In horses administered dalteparin once daily, anti-factor Xa activity fell below the suggested thromboprophylactic range (~16 hours after administration (Fig 2; Table 1). In contrast when dalteparin was administered twice daily, anti-factor Xa activity was maintained within or above this suggested range for the entire 24 hour period (Fig 2; Table 1).

The values for anti-factor Xa activity we report are higher than those reported previously.\(^{17}\) As a result, the pharmacodynamic variables reported here are quite different to those reported earlier.\(^{22}\) These differences might be, at least in part, explained by the use of different anti-factor Xa assays in the current and previous studies.\(^{17,22}\) A number of manufacturers have produced chromogenic assays for the measurement of anti-factor Xa activity that can be used in a variety of automated coagulation analyzers. The use of different assays and/or automated analyzers can produce clinically significant differences in reported anti-factor Xa activity.\(^{27,28}\) The specific chromogenic assay used in the 2 earlier studies and the current study were different in each case and the coagulation analyzer used in the current study was also different from that used in previous studies.

An earlier study in horses demonstrated that anti-factor Xa activity fell below a range suggested for thromboprophylaxis (0.1–0.2 U/mL) between 12 and 24 hours after administration of a single 50 U/kg dose of dalteparin.\(^{22}\) We confirmed this; pharmacodynamic analysis revealed that plasma anti-factor Xa

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**Figure 2** Mean ± SD plasma anti-factor Xa activity over time in healthy horses administered LMWH (50 U/kg) subcutaneously either once (A) or twice (B) daily. The left panel shows anti-factor Xa activity during the first 24 hours of the study. The right panel shows anti-factor Xa activity for the 24 hours following the last dose (i.e., at 48 hours for once daily LMWH administration and 60 hours for twice daily administration). The horizontal dashed line indicates a plasma anti-factor Xa activity that has been suggested for thromboprophylaxis in humans (0.1 U/mL). The black arrow indicates the time point at which the second dose of LMWH was administered in the group receiving LMWH every 12 hours.
Low molecular weight heparin was administered either every 24 hours or every 12 hours for 3 consecutive days.

$C_{\text{max}}$, peak plasma anti-factor Xa activity; $T_{\text{max}}$, time to maximum anti-factor Xa activity; $t_{\text{1/2}}$, elimination half-life; $\text{AUC}_{0-12\text{h}}$, area under the plasma anti-factor Xa activity versus time curve for the first 12 hours; $\text{AUC}_{0-\infty}$, area under the plasma anti-factor Xa activity versus time curve extrapolated to infinity; $\text{MRT}$, mean residence time; $V_{d_{\text{area}}}/F$, apparent volume of distribution (area) divided by bioavailability (unknown); $CL/F$, clearance divided by bioavailability (unknown); $T > 0.1 \text{ U/mL}$, time for which plasma anti-factor Xa activity remained above 0.1 U/mL after achieving steady state; NA, not applicable.

Significant effect of frequency of administration (12 hours versus 24 hours) on a given variable ($P < .05$).

*Significant effect of sampling time (i.e., first versus last LMWH dose) on a given variable ($P < .05$).

Median and range.

Table 1: Mean ± SD Pharmacodynamic Variables of Plasma Anti-Factor Xa Activity After Subcutaneous Administration of an LMWH Formulation (50 U/kg) to 6 Healthy Adult Horses

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dose</th>
<th>Frequency of Administration [hr]</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (U/mL)</td>
<td>First</td>
<td>0.61 ± 0.29, 0.58 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>Last</td>
<td>0.91 ± 0.47, 0.96 ± 0.14</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>First</td>
<td>3 (1–6), 3 (1–6)</td>
</tr>
<tr>
<td></td>
<td>Last</td>
<td>4.78 ± 2.90, 6.00 ± 3.06</td>
</tr>
<tr>
<td></td>
<td>Last</td>
<td>6.83 ± 5.19, 6.03 ± 3.05</td>
</tr>
<tr>
<td>$t_{\text{1/2}}$ (h)</td>
<td>First</td>
<td>6.01 ± 1.77*, 4.74 ± 1.07†</td>
</tr>
<tr>
<td></td>
<td>Last</td>
<td>7.24 ± 2.54, 8.66 ± 1.55</td>
</tr>
<tr>
<td>$\text{AUC}_{0-12\text{h}}$ (U·h/mL)</td>
<td>First</td>
<td>7.62 ± 2.01*, 7.08 ± 2.32†</td>
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<tr>
<td></td>
<td>Last</td>
<td>10.76 ± 3.17, 12.67 ± 4.7</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (U·h/mL)</td>
<td>First</td>
<td>8.49 ± 3.49, 10.10 ± 4.38</td>
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<tr>
<td></td>
<td>Last</td>
<td>11.55 ± 7.61, 9.95 ± 3.79</td>
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<tr>
<td>$V_{d_{\text{area}}}/F$ (mL/kg)</td>
<td>First</td>
<td>45.67 ± 26.29, 61.20 ± 20.22</td>
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<tr>
<td></td>
<td>Last</td>
<td>43.76 ± 25.45, 33.02 ± 6.66</td>
</tr>
<tr>
<td>$CL/F$ (mL/h/kg)</td>
<td>First</td>
<td>6.93 ± 1.72*, 7.67 ± 2.31†</td>
</tr>
<tr>
<td></td>
<td>Last</td>
<td>5.01 ± 1.51, 4.45 ± 1.65</td>
</tr>
<tr>
<td>$T &gt; 0.1 \text{ U/mL}$ (h)</td>
<td>First</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Last</td>
<td>15.9 ± 2.8*, 24.0 ± 0.0b</td>
</tr>
</tbody>
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Pharmacodynamics of Multi-Dose LMWH in Horses

activity remained above 0.1 U/mL for ~16 hours in horses treated once daily (Table 1). Increasing the dose of dalteparin to 100 U/kg appeared to extend the time that anti-factor Xa activity exceeded 0.1 U/mL in healthy horses but was not able to maintain activity above this level for the entire 24 hour dosing interval. We have shown that anti-factor Xa activity remains within or above the thromboprophylactic range for the entire 24 hours in horses administered dalteparin (50 U/kg) twice daily (Fig 2, Table 1). Based on these findings and the suggestion from human studies that insufficient factor Xa inhibition might increase the risk of thrombosis, administration of dalteparin every 12 hours might be desirable in adult horses. However, the same human study also indicated that increases in bleeding complications occurred when anti-factor Xa activity exceeded 0.2 U/mL 12 hours after administration.

This study was performed on healthy horses and the pharmacodynamics of LMWH might be quite different in critically ill horses. In a clinical study evaluating the efficacy of dalteparin in horses with colic, the incidence of jugular vein abnormalities was significantly lower in horses treated with LMWH when compared with horses treated with UFH. Despite this apparent clinical efficacy, mean anti-factor Xa activity did not increase significantly from baseline in the sickest horses. Adequate anti-factor Xa activity (i.e., >0.1 U/mL) is achieved 3 hours after administration of a single dose of dalteparin (100 U/kg) to healthy equine neonates. In contrast, mean anti-factor Xa activity was considered inadequate in septic foals after administration of the first 2 doses of dalteparin (100 U/kg every 24 hours) and adequate factor Xa inhibition was only achieved in 4 of 6 foals during the study. Similar results have been described in critically ill people in whom standard LMWH dosing regimens often fail to achieve adequate factor Xa inhibition. Despite the claim that the pharmacodynamics of LMWH allows infrequent monitoring, frequent measurement of peak and trough anti-factor Xa activity and dose adjustment appears necessary in some critically ill human trauma and surgical patients and the same might be true for critically ill horses.

There are some limitations to our study that should be considered when interpreting the results. The SD about the means for anti-factor Xa activity tended to be large (Fig 2). Possible reasons for this include the small number of horses, variability in the drug response of individual horses and variation in the measurement of plasma anti-factor Xa activity. Age, percent lean body mass, reproductive status, and renal function have been suggested to affect the pharmacodynamics of LMWH in people. Age reportedly affects dalteparin pharmacodynamics in horses. Although none of the mares used were pregnant, they were likely in different stages of the estrus cycles during the study and this might have affected results. Prolonged catheterization is also...
pro-thrombotic and might have confounded results of coagulation testing. Additional, unknown physiologic factors might affect drug responses in individual horses resulting in the large variations in anti-factor Xa activity we observed. Intra-assay coefficient of variation ranged between 0% and 8.1% in this study and assay variation might have also contributed to the wide standard deviations observed. Only a single LMWH formulation and a single dose (50 U/mL) were assessed. Unfortunately, the expense of LMWH formulations precluded evaluation of multiple formulations and doses. Dalteparin is the LMWH formulation that has been most studied in horses\textsuperscript{17,22} and is the formulation that has been most often evaluated clinically.\textsuperscript{18,24} The LMWH formulation enoxaparin has also been evaluated in healthy horses; the pharmacodynamic and anti-coagulant properties of enoxaparin in horses were similar to those found in people.\textsuperscript{22} Enoxaparin might also have some benefit in the prevention of laminitis.\textsuperscript{23}

We concluded that once daily administration of dalteparin does not maintain anti-factor Xa activity above the lower limit of the recommended thromboprophylactic range for the entire dosing interval. In contrast, thromboprophylactic levels were maintained for 24 hours with twice daily dalteparin dosing. However, maintaining of higher anti-factor Xa activity might increase the risk of bleeding complications although this was not evident; further investigation of dosing regimens are warranted particularly in critically ill horses. Despite a significant increase in the AUC from the first to last dose with attainment of steady-state activity, no clinically relevant adverse effects were detected after once or twice daily administration of dalteparin for 3 consecutive days. Considerable variability in anti-factor Xa activity between horses was evident and might indicate the need for more frequent monitoring than suggested in human medicine.

REFERENCES

30. de Toledo JS, Gunawardena S, Munoz R, et al: Do neonates, infants and young children need a higher dose of enoxaparin in the cardiac intensive care unit? *Cardiol Young* 2010;20:138–143