Comparison of pharmacokinetic variables for two low-molecular-weight heparins after subcutaneous administration of a single dose to horses

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Objective—To determine pharmacokinetic variables and to evaluate the influence on clotting times after SC administration of single doses of dalteparin and enoxaparin to horses.

Animals—5 healthy adult horses.

Procedures—The study was designed as a 4-period crossover study. Each horse received a single SC injection of dalteparin (50 and 100 anti-Xa U/kg) and enoxaparin (40 and 80 anti-Xa U/kg). Plasma anti-Xa activities and clotting times were measured, and pharmacokinetic variables were determined. Absolute and relative maximal prolongation of clotting times was calculated, and correlation between plasma anti-Xa activities and clotting times was determined.

Results—The SC administration of each of the doses of the 2 preparations was well tolerated. Time course of the anti-Xa activities could be described in a 1-compartment model. Comparison of low- and high-dose treatments revealed a disproportionate increase of the area under the plasma activity-time curve and prolongation of the terminal half-life, but the increase in maximum plasma activity was proportionate, and peak plasma concentrations corresponded with concentrations recommended in human medicine. There were only mild changes in activated partial thromboplastin time (aPTT), whereas the influence on thrombin time (TT) was greater, dose-dependent, and more variable. A weak-to-moderate correlation between aPTT and plasma anti-Xa activities and a moderate-to-strong correlation between TT and plasma anti-Xa activities were found.

Conclusions and Clinical Relevance—Pharmacokinetic and anticoagulatory properties of low-molecular-weight heparins in horses are similar to those found in humans. Once-daily SC administration of dalteparin or enoxaparin may be useful as an anticoagulatory treatment in horses. (Am J Vet Res 2002; 63:868–873)
between treatments. Each horse received a single SC injection of each of 4 treatments: dalteparin, 50 and 100 anti-Xa U/kg (low and high doses, respectively); and enoxaparin, 40 and 80 anti-Xa U/kg (low and high doses, respectively).

Collection of blood samples—Before administration of LMWH, a catheter was inserted in a jugular vein for use in collection of blood samples. The catheter was left in place for the duration of each trial and was flushed with saline (0.9% NaCl) solution after collection of each sample. Venous blood samples were collected before and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 12, 14, 16, and 24 hours after each LMWH injection. In addition, blood samples were collected 0.25 hour after injection of the low doses of each LMWH and 5.5, 9, 11, and 13 hours after injection of the high doses of each LMWH. Blood samples were collected into tubes containing sodium citrate and then were centrifuged at 4,000 × g for 15 minutes. Plasma was apportioned into 2-ml aliquots, snap-frozen in liquid nitrogen, and stored at –73 C until analysis.

Laboratory analysis—Plasma anti-Xa activity was measured by use of a chromogenic assay using an automated analyzer. A calibration curve was established by use of a pool of citrated plasma collected from 30 healthy horses that was spiked with increasing amounts of dalteparin (0, 0.2, 0.4, and 0.8 anti-Xa U/ml). Quality control was performed by use of 2 dilutions of the LMWH preparations with defined anti-Xa activities (0.2 and 0.4 anti-Xa U/ml). Coagulation variables were determined by use of an automated analyzer. Activated partial thromboplastin time (aPTT) was measured by use of a coagulometric test kit. Reagent for the measurement of thrombin time (TT) was prepared, using 5,000 units of bovine thrombin in 2 ml of distilled water, 43 ml of veronal-acetate buffer (pH 7.39), 43 mg of bovine serum albumin, and 5 ml of glycerin.

Pharmacokinetic calculations and data analysis—The time course of plasma anti-Xa activity was assessed by use of a semilogarithmic graphic presentation. Pharmacokinetic variables were determined by use of standard methods for each horse, using a 1-compartment model. The rate constants β and α as well as the extrapolated initial concentrations B and A were calculated by use of linear regression analysis. The terminal half-life (t1/2β) and absorption half-life (t1/2α) were obtained by use of the equations t1/2β = (ln 2)/β and t1/2α = (ln 2)/α. Maximum plasma activity (Cpmax) and time until Cpmax (tmax) were calculated by use of the equations tmax = (ln [Bα]/(β – α)) and Cpmax = B • eCpmax – eCpmax). Area under the plasma-activity time curve (AUC) was determined by use of variables from the 1-compartment model (AUCtrap = 1/βB) and the model-independent trapezoid method (AUCmodel, respectively). For calculation of values for AUCtrap, the terminal part of the curve (time > 24 hours) was extrapolated by use of the last 3 data points of the plasma activity-vs-time curve.

Apparent total body clearance (ClT) was calculated as a ratio for the injected dose to the AUC, and the apparent volume of distribution (Vd) was calculated by use of the equation Vd = ClT/β. Values for ClT and Vd were determined by use of both the AUCtrap and AUCmodel, assuming bioavailability (F) was 100%.

Reference values for aPTT (36.7 to 57.7 seconds) and TT (14.2 to 25.2 seconds) have been reported elsewhere. Mean ± SD maximal prolongation in clotting times (Aα/A0) was calculated on the basis of differences between maximal measured clotting times (A0) and baseline values (Aα) of each horse. Maximal prolongation relative to baseline values was calculated as Aα/A0:

All calculations were performed by use of a computer software package. Data were analyzed by use of graphic presentation and descriptive statistics (mean ± SD). The Pearson correlation between plasma anti-Xa activities and clotting times was determined. Values of P ≤ 0.05 were considered significant.

Results

Clinical observations—Subcutaneous administration of the various preparations was well tolerated by the horses. Clinical abnormalities or local reactions at each injection site were not observed during the study.

Pharmacokinetics—Time courses of the mean measured plasma anti-Xa activities were similar after injection of both doses of dalteparin and enoxaparin (Fig 1). Analysis of semilogarithmic graphs of the plasma activity-vs-time curves did not suggest a redistribution phase, and the curves were adequately described by a 1-compartment model in all cases. Ranges of values for the AUCtrap and AUCmodel were similar (Table 1). Comparison of variables after administration of low and high doses revealed a disproportionate increase in the AUC and prolongation of t1/2β with higher doses. However, variation of AUC and t1/2β was high. Variation of Cmax was small, and Cmax had a proportionate increase with doubling of the dose. Values for t1/2α were similar for administration of low and high doses. The tmax was slightly delayed for administration of the high doses. The ClT was slightly decreased for administration of high-dose LMWH, although the differences in the ranges were small. The Vd were within similar ranges for all doses.

Compared with values for dalteparin, t1/2β of enoxaparin was slightly prolonged and ClT was smaller. The t1/2α of enoxaparin was shorter than t1/2α of dalteparin. Anti-Xa activities 12 hours after injection of low and high doses of dalteparin were 0.06 ± 0.06 and 0.24 ± 0.10 U/ml, respectively, whereas values after injection of low and high doses of enoxaparin were 0.06 ± 0.04 and 0.25 ± 0.03 U/ml, respectively. Substantial residual
anti-Xa activity was still evident 24 hours after SC injection of the high dose of each LMWH (dalteparin, 0.06 ± 0.04 U/ml; enoxaparin, 0.07 ± 0.05 U/ml).

Clotting times—Subcutaneous injection of LMWH had only a small effect on aPTT for all 4 treatments (Table 2). All values remained within the reference range. The influence on TT was stronger, dose-dependent, and more variable. In high-dose treatments of both LMWH, the TT markedly exceeded the upper value for the reference range. The TT was more prolonged after administration of dalteparin, compared with administration of enoxaparin. Twenty-four hours after administration of either LMWH, aPTT and TT were within baseline ranges. Correlation analysis between plasma anti-Xa activities and clotting times revealed a weak-to-moderate correlation for aPTT, which was not significant for the low-dose enoxaparin treatment, and a moderate-to-strong correlation for TT (Table 3).

### Discussion

Unfractionated heparin is composed of a heterogeneous mixture of highly sulfated polysaccharide chains with variable length and molecular weights that range from 3 to 30 kd.\(^\text{13,14}\) Low-molecular-weight heparins are fragments of UFH that are produced by chemical or enzymatic depolymerization.\(^\text{1,2}\) Pharmacokinetic properties and inhibitory activity of UFH and LMWH on factor Xa and factor IIa vary, depending on the chain length and molecular-weight distribution of the heparin molecules.\(^\text{7,13,14}\) Unfractionated heparin prepared by extraction from porcine intestinal mucosa has a mean molecular weight of 12 to 15 kd and a ratio for anti-Xa activity to anti-IIa activity (anti-Xa:anti-IIa) of 1.0, whereas dalteparin prepared by nitrous acid depolymerization has a mean molecular weight of 5 to 6 kd and an anti-Xa:anti-IIa ratio of 2.7, and enoxaparin prepared by benzylamine and alkaline polymerization has a mean molecular weight of
4.2 kd and an anti-Xa:anti-IIa of 3.8. Studies in which investigators administered various LMWH to healthy volunteers have established that LMWH are not bioequivalent when comparing equivalent anti-Xa doses. Consequently, different doses of various LMWH are recommended for clinical indications in human medicine on the basis of fixed-dose SC administration on a daily basis. When administered at these doses, anti-Xa activity of dalteparin and enoxaparin can be considered bioequivalent.

Recommended doses for prophylaxis of thrombosis and coagulation disorders in humans depend on the relative risk for developing thromboembolic disorders. A low-dose treatment is used for prophylactic purposes in most human patients, whereas a high-dose treatment is used for patients with a known risk for venous thrombosis or for treatment of patients with existing thrombosis. In the study reported here, dalteparin and enoxaparin were chosen for use in investigating 2 LMWH with distinct molecular weights and anticoagulant properties that are commonly used in humans. Dose recommendations based on pharmacokinetic studies currently are not available for horses. However, data from other investigations indicate that a dosage of 50 U of dalteparin/kg may be adequate for prophylactic use in horses, comparable to low-dose LMWH therapy in humans. On the basis of results obtained in those studies in horses and recommendations in human medicine, dosages of 50 and 100 U of dalteparin/kg and 40 and 80 U of enoxaparin/kg were chosen as low- and high-dose treatments in the study reported here.

Mean anti-Xa activities 3, 12, and 24 hours after a single SC injection of low-dose dalteparin in our study were similar to the activities detected in 2 other studies in horses. Similar to results of studies in humans, the time course of the anti-Xa activities in the study reported here could be described by a 1-compartment model.

Clear dose-dependent pharmacokinetics for UFH have been found in other investigations in humans and horses. In contrast to UFH, the pharmacokinetics of LMWH in humans are considered to be linear and stationary. The prolongation of the TT was greater than described in other investigations. The difference in prolongation of TT found between dalteparin and enoxaparin is likely to be attributable to the differing anti-Xa:anti-IIa of the 2 preparations, reflecting the stronger inhibition of factor IIa by dalteparin, compared with enoxaparin.

Bioavailability of LMWH in horses was not determined in the study reported here, because the time course of the plasma anti-Xa activity after IV administration was not measured. Results from studies in horses indicate that F of LMWH is dose-independent and at least as high as 90% after SC administration. The F of LMWH in horses is expected to be within a corresponding range, because the other pharmacokinetic variables were comparable to those found in humans.

The influence of LMWH treatment on the aPTT of horses was minimal in the study reported here and similar to results of other studies in humans and horses. Because aPTT did not exceed the upper value of the reference range for any of the 4 treatments, the effect of LMWH on aPTT in healthy horses does not seem to be biologically important at the dosages used. However, the prolongation of the TT was greater than described in other investigations. The difference in prolongation of TT found between dalteparin and enoxaparin is likely to be attributable to the differing anti-Xa:anti-IIa of the 2 preparations, reflecting the stronger inhibition of factor IIa by dalteparin, compared with enoxaparin.

Correlation between heparin concentration (anti-Xa activity) and clotting times is weak in horses and humans. Tests currently recommended for monitoring of LMWH therapy in humans and horses should not be used for monitoring of LMWH therapy in horses. Tests currently recommended for monitoring of LMWH therapy in humans and horses that measure anti-Xa activity in plasma. However, in humans, the incidence of adverse effects with LMWH therapy is low, pharmacokinetics are dose-independent and predictable, and adequate anticoagulation is possible with fixed-dose administration in most patients.
Therefore, the use of each of these agents does not require dosage adjustments on the basis of results of coagulation studies. In accordance with our current knowledge of the clinical and pharmacologic properties of LMWH in horses, these recommendations may also be adopted for use in horses.

Use of LMWH in horses is considered to have fewer adverse effects, longer half-inactivation time, and more predictable anticoagulatory effects, compared with use of UFH. Doses of 50 U of dalteparin/kg, SC, q 24 h or 40 U of enoxaparin/kg, SC, q 24 h seem to be adequate for prophylactic anticoagulatory treatment of horses. For treatment of coagulation disorders or for ill horses that are considered to be at high risk for developing thrombotic disease, dosages may need to be increased to 100 U of dalteparin/kg, SC, q 24 h or 80 U of enoxaparin/kg, SC, q 24 h, respectively.


Fragmin, 100,000 anti-Xa U/4 ml, Pharmacia & Upjohn AG, Dubendorf, Switzerland.

Clexane multi, 30,000 anti-Xa U/ml, Aventis Pharma, Zurich, Switzerland.

Secalon T, Becton Dickinson, Basel, Switzerland.

Natrum chlorata 0.9%, Laboratorium Dr. G. Bichsel AG, Interlaken, Switzerland.

BD Vacutainer, Becton Dickinson Vacutainer Systems Europe, Plymouth, UK.

Cumarin heparin, Endotell AG, Allschwil, Switzerland.

Cobas Mira, Roche, Basel, Switzerland.

BCS coagulation analyzer, Dade Behring AG, Dudingen, Switzerland.

Dade Actin FS activated PTT reagent, Dade Behring Marburg GmbH, Marburg, Germany.

Bovine thrombin, Diagnostec AG, Liestal, Switzerland.


Microsoft Excel 2000, Microsoft Corp, Redmond, Wash.

StatView 5.0 for Windows, SAS Institute Inc, Cary, NC.

References