Assessment of the effects of dalteparin on coagulation variables and determination of a treatment schedule for use in cats

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OBJECTIVE
To determine a treatment protocol for SC administration of dalteparin to cats on the basis of currently available detailed pharmacokinetic data and to assess the effect of SC administration of dalteparin to cats on coagulation variables such as activated partial thromboplastin time (aPTT), thrombin time, and results for thromboelastometry, compared with effects on anti–activated coagulation factor X (anti-Xa) activity.

ANIMALS
6 healthy domestic shorthair cats.

PROCEDURES
Cats received 14 injections of dalteparin (75 anti-Xa U/kg, SC) at 6-hour intervals. Blood samples were collected before and 2 hours after the first and second injections on days 1, 2, and 4. Anti-Xa activity was measured by use of a chromogenic substrate assay, aPTT and thrombin time were measured by use of an automated coagulometer, and viscoelastic measurements were obtained with thromboelastrometry.

RESULTS
2 hours after the second injection, the target peak anti-Xa activity range of 0.5 to 1.0 U/mL was achieved in all cats, whereas median trough values remained below this range. Peak anti-Xa activity had only minimal effects on coagulation variables; the maximum median ratio for aPTT (in relationship to the value before the first dalteparin injection) was 1.23.

CONCLUSIONS AND CLINICAL RELEVANCE
Results of this study indicated that this treatment protocol resulted in reproducible anti-Xa activity in cats that was mostly within the targeted peak range of anti-Xa activity recommended for humans. Treatment in accordance with this protocol may not require routine coagulation monitoring of cats, but this must be confirmed in feline patients. (*Am J Vet Res* 2016;77:700–707)

In human patients, LMWHs are considered more convenient and at least as effective as UFH[1,2] and have replaced UFH as the standard anticoagulant for use in thromboprophylaxis and treatment of acute thrombosis.[3] The SC administration of LMWHs results in high bioavailability and more predictable heparin plasma (anti-Xa) activities, which makes routine monitoring in most human patients unnecessary, in contrast to monitoring required after the use of UFH.[4] In addition, the long terminal half-life of LMWHs in humans allows long intervals between doses.[5]

ABBREVIATONS

anti-Xa	Anti–coagulation factor Xa
aPTT	Activated partial thromboplastin time
CT	Clotting time
LMWH	Low-molecular-weight heparin
TEM	Thromboelastometry
TT	Thrombin time
UFH	Unfractionated heparin

Variable dosages of LMWHs have been used in cats for treatment or prevention of thromboembolic events.[6,7,a] Studies have involved the use of various treatment protocols for dalteparin and other LMWHs,[8–10,b] but investigators, with the exception of 1 study[b] failed to achieve assumed peak anti-Xa activities within the target range. However, blood samples in all these studies were collected 4 hours after injection; 4 hours is the time of maximum drug activity in humans.[3]

Pharmacokinetic studies[11,c] revealed that cats have rapid absorption and elimination of LMWH and that peak concentrations are reached by approximately 2 hours after SC administration of dalteparin. Therefore, the objective of the study reported here was to evaluate a dosage schedule for body weight–adjusted SC administration of dalteparin to cats, with the dosage schedule calculated on the basis of currently available detailed pharmacokinetic data. A second objective was to assess pharmacodynamic effects of administration of daltepa-

rin in accordance with this treatment schedule on various coagulation tests.

Materials and Methods

Animals

Six healthy domestic shorthair cats (5 neutered males and 1 neutered female) were included in the study. Cats were between 1 and 10 years of age (median, 7.5 years) and weighed between 4.3 and 6 kg (median, 4.9 kg). Cats were considered healthy on the basis of daily clinical examinations conducted throughout the study and hematologic status (CBC and biochemical profile) determined prior to the first dalteparin injection. Values measured before the start of the study for all hemostasis tests were within institutional reference intervals.

Cats typically were housed as a group at the Institute for Parasitology of the University of Veterinary Medicine Hannover. During the experiment, cats were housed separately. Food was withdrawn 12 hours before the first heparin injection and returned after collection of the last blood sample of the day; water was available ad libitum throughout the experiment. Animals in this study were used in accordance with German Animal Welfare law. The experimental design was approved by the official Animal Health Care Officer of the university and the Ethics Committee of the Lower Saxony State Office for Consumer Protection and Food Safety.

Study design

On the basis of pharmacokinetic data obtained after a single SC injection of dalteparin in a previous study,[11] a treatment protocol for dalteparin was determined and then evaluated. Each of the 6 cats received injections of dalteparin sodium (75 anti-Xa U/kg, SC, q 6 h) for 4 consecutive days (a total of 14 injections because the experiment was terminated after the second LMWH injection on day 4). The first day of injection was designated as day 1, and time of the first injection on day 1 was designated as time 0.

Blood samples were collected immediately before and 2 hours after (ie, the time of maximum blood concentrations of dalteparin) the first and second injections on days 1, 2, and 4; thus, blood samples were collected at 0 (baseline), 2, 6, 8, 24, 26, 30, 32, 72, 74, 78, and 80 hours (total of 12 blood samples). Samples were used to determine baseline, peak, and trough activities of dalteparin. Each blood sample was analyzed to determine anti-Xa activity, aPTT (with 2 reagents), TT (with 2 thrombin concentrations), TEM variables, antithrombin activity, Hct, and platelet counts.

Collection and preparation of blood samples

Blood samples were collected from a cephalic or femoral vein by use of 20-gauge, 1.5-inch needles. Blood samples were collected directly into sample tubes. Approximately 0.5 mL of blood was collected into an EDTA-containing tube and used to determine Hct and platelet counts. In addition, two 1.3-mL plastic tubes[d] containing 0.11 mol/L (3.8%) sodium citrate were filled to the mark (1 part sodium citrate: 9 parts blood) and used for coagulation tests and TEM. Immediately after collection of each sample, tubes were gently rocked to thoroughly mix the blood and anticoagulant.

Samples were stored at room temperature (approx 22°C) for approximately 30 minutes. Then, 2 aliquots (300 µL/aliquot) of citrated blood were used for TEM assays. The remaining citrated blood was centrifuged with a microcentrifuge (16,000 × *g* for 10 minutes at room temperature). Plasma was harvested and transferred into other plain plastic tubes, and centrifugation was repeated with these tubes. The final platelet-free plasma was then divided into small aliquots (1 aliquot of 250 µL and 3 aliquots of 200 µL each) and frozen at –70°C until used for analysis. Immediately before analysis, platelet-free plasma was thawed at 37°C in a water bath.

Laboratory analysis

Amidolytic anti-Xa activity was measured by use of a chromogenic substrate test[e] in an autoanalyzer[f] in accordance with the manufacturer's instructions. Standard solutions (0, 0.25, 0.5, 0.75, and 1 anti-Xa U/mL) were prepared by use of various dilutions of the dalteparin preparation used in the experiment[g] and pooled plasma obtained from 25 clinically normal cats.

The aPTT and TT were measured with an automated coagulation analyzer[h]; the technique was based on a spheric coagulometric method. Two commercial aPTT reagents were used: reagent 1[i] ($aPTT_{R1}$), which was reconstituted in accordance with the test manual, and reagent 2[j] ($aPTT_{R2}$). The TT assay was performed with a commercial reagent containing bovine thrombin[k] at each of 2 thrombin concentrations (1.5 and 3 U/mL), which resulted in final thrombin concentrations of 1 U/mL (TT_{1U}) and 2 U/mL (TT_{2U}). In addition to the raw data (coagulation times), results of aPTT and TT for each time point were reported as ratios (in relationship to baseline values obtained at time 0).

The TEM analyses for each sample were performed in parallel on 2 channels of a TEM instrument,[l] with (20 µL of calcium solution,[m] 20 µL of kaolin solution[i] [5 g/L], and 300 µL of citrated blood) and without (20 µL of calcium solution,[m] 20 µL of isotonic saline [0.9% NaCl] solution instead of kaolin solution, and 300 µL of citrated blood) activation. Fully automatic pipettes were used to minimize ratio errors, and the registration time (test cycle) was set to 3,600 seconds. Variables provided automatically by the instrument included CT, clot formation time, α angle, and maximum clot firmness. The CT assays that did not have detectable coagulation within 3,600 seconds were censored, and statistical calculations were performed with a time of 3,600 seconds for those assays. In addition, CT values were converted to ratios.

Antithrombin activity was measured by use of a chromogenic thrombin-dependent substrate test[n] in the autoanalyzer[f] in accordance with the manufacturer's instructions. Calibration was performed with pooled plasma obtained from 25 clinically normal cats (defined as 100% activity) and isotonic saline solution (defined as 0% activity).

The Hct and platelet counts were measured automatically.[o] The blood cell counter used isovolumetric sphering followed by a double-angle laser light–scattering procedure to detect platelets and RBCs.

Statistical analysis

All data were tested for normal distributions by use of the Kolmogorov-Smirnov test. Regardless of a normal distribution, all descriptive data were reported as median and range and graphically illustrated as box-and-whisker plots to provide homogeneous reporting of data. Normally distributed data were compared with a repeated-measures ANOVA; data that were not normally distributed were compared with the Friedman test. When significant differences were detected, all time points were tested against the baseline value (paired *t* test for parametric analysis or Wilcoxon rank sum test for nonparametric analysis). Ratios calculated for aPTT, TT, and CT of the TEM analysis and that especially represented the anticoagulatory effect of LMWH were compared among time points by use of an ANOVA and *t* tests for independent observations if data were normally distributed. Only CT for TEM assays without activation that included censored data were compared with the Kruskal-Wallis test, and post hoc tests were performed with the Mann-Whitney *U* test. Spearman rank correlation coefficients were calculated for the relationship between anti-Xa values (only samples with anti-Xa activity > 0.05 U/mL were included) and ratios of aPTT, TT, and CT. For all statistical tests, values of $P < 0.05$ were considered significant. The α adjustment for multiple comparisons was waived to avoid too conservative and nonplausible post hoc test results in relation to results of global statistical comparisons. Analytical software programs[p,q] were used for the statistical analyses.

Results

Adverse effects

Increased hemorrhage from venipuncture sites was observed at times of peak anti-Xa activity. This hemorrhage led to development of hematomas, despite application of moderate pressure with a bandage. No episodes of spontaneous hemorrhage were observed in the cats.

Anti-Xa activity

The target peak anti-Xa activity range of 0.5 to 1.0 anti-Xa U/mL was achieved by 2 hours after the second injection in all cats; the target range was exceeded at least once in 5 cats and reached values of up to 1.3 anti-Xa U/mL **(Table 1)**. After the second dalteparin injection was administered, peak activities did not have a cumulative effect ($P = 0.382$; repeated-measures ANOVA including data for 8, 26, 32, 74, and 80 hours). In contrast, trough values remained below the target range during the experiment.

Coagulation tests

There were significant ($P < 0.001$; repeated-measures ANOVA) changes for $aPTT_{R1}$ throughout the experimental period. All time points with peak anti-Xa activity (2 hours after injections) were significantly (paired *t* test) prolonged, compared with the baseline value, whereas no time point with trough heparin activities had a significantly altered $aPTT_{R1}$. Statistical analysis of ratios revealed a similar pattern **(Figure 1)**. The maximum median ratio for $aPTT_{R1}$ was 1.23. In contrast, results of global comparison for $aPTT_{R2}$ did not indicate significant changes among time points for the original data ($P = 0.095$; repeated-measures ANOVA) or for ratios ($P = 0.166$; ANOVA; results not shown).

There were significant ($P < 0.001$) changes over time for the TT_{1U} (repeated-measures ANOVA) and TT_{2U} (Friedman test; Table 1). The TT_{1U} was significantly prolonged, compared with the baseline value, at all peak anti-Xa times except for the last time (Figure 1). In contrast, TT_{2U} was significantly ($P < 0.05$; Wilcoxon rank sum test) prolonged only at the first 2 peak time points.

Ratios for TT_{1U} also changed significantly ($P < 0.001$; ANOVA) over time, but ratios were significantly (*t* test) increased only at 3 peak time points, with a maximum median value of 1.19 (Figure 1). Ratios for TT_{2U} did not change significantly ($P = 0.115$; Kruskal-Wallis test) over time.

TEM

Individual TEM assays without activation did not result in a detectable clot at various peak time points (8/36 measurements for 4 cats). Significant ($P < 0.001$) changes among time points were detected for CT without activation (Friedman test) and with kaolin activation (repeated-measures ANOVA). Significant (Wilcoxon rank sum test) prolongation of CT without activation, compared with baseline values, was limited to specific peak time points, whereas CT with kaolin activation was significantly prolonged at all peak time points (Table 1). Appropriately, ratios for both CTs changed significantly (CT without activation, $P = 0.005$; Kruskal-Wallis test; CT with kaolin activation, $P < 0.001$; ANOVA) over time, and all peak time points had significant increases in ratios **(Figure 2)**.

Analysis of the remaining results of kaolin-activated TEM revealed significant changes (repeated-measures ANOVA) among time points for clot formation time ($P < 0.001$), α angle ($P = 0.047$), and maximum clot firmness ($P < 0.001$). Post hoc analyses comparing individual time points with baseline values revealed a significant increase in clot formation time and decrease of α angle at 4 of 6 peak times, whereas no

Table 1—Values for anti-Xa activity, aPTT measured with reagent 1 ($aPTT_{R1}$) or regeant 2 ($aPTT_{R2}$), TT measured with a final thrombin concentration of 1 U/mL (TT_{1U}) or 2 U/mL (TT_{2U}), and TEM variables in samples obtained from 6 healthy cats receiving dalteparin (75 anti-Xa U/kg, SC, q 6 h for 4 days).

Variable		0 hours*	2 hours†	6 hours*	8 hours†	24 hours*	26 hours†	30 hours*	32 hours†	72 hours*	74 hours†	78 hours*	80 hours†
Anti-Xa activity (U/mL)	Median	0	0.77	0.17	0.80	0.28	0.89	0.33	0.81	0.44	0.89	0.32	1.04
	Range	0–0.03	0.41–0.98	0.07–0.22	0.71–0.90	0.21–0.46	0.54–1.11	0.20–0.42	0.57–1.12	0.25–0.53	0.69–1.17	0.20–0.51	0.66–1.30
	P value	—	< 0.001	0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001
$aPTT_{R1}$ (s)	Median	12.3	14.6	12.4	14.8	12.3	14.7	12.3	14.8	11.9	14.1	12.1	14.5
	Range	10.0–13.2	13.3–15.4	11.6–13.4	13.7–15.6	11.3–12.8	14.2–15.9	11.3–13.2	14.1–15.6	10.5–12.4	13.2–15,2	11.0–12.8	14.1–15.1
	P value	—	0.002	0.109	< 0.001	0.576	< 0.001	0.152	< 0.001	0.521	0.005	0.765	0.005
$aPTT_{R2}$ (s)	Median	19.2	23.3	23.1	29.4	21.4	24.4	22.5	26.0	19.0	22.6	20.2	19.9
	Range	15.9–32.2	21.2–29.8	16.9–30.9	21.2–43.8	17.0–34.5	15.4–41.1	16.3–33.8	18.6–44.9	17.6–46.0	22.2–41.7	18.8–34.9	18.7–53.2
	P value	—	—	—	—	—	—	—	—	—	—	—	—
TT_{1U} (s)	Median	14.7	18.9	14.9	17.7	14.5	14.5	14.7	17.8	14.5	16.5	14.7	16.9
	Range	8.1–17.3	9.0–19.9	7.7–17.7	9.0–20.9	7.9–17.8	8.4–22.6	8.0–17.7	9.0–21.6	8.0–16.8	8.8–19.3	8.0–17.3	9.1–19.8
	P value	—	0.002	0.488	0.003	0.427	0.010	0.732	0.020	0.034	0.003	0.497	0.060
TT_{2U} (s)	Median	8.0	9.7	8.2	9.3	8.7	9.5	8.2	9.4	7.9	9.0	7.9	8.9
	Range	7.2–15.3	8.1–17.1	7.6–15.0	7.8–17.4	7.2–15.0	7.5–16.0	7.2–14.0	7.5–15.9	7.0–14.0	7.7–16.0	7.1–13.6	7.6–15.8
	P value	—	0.031	0.223	0.041	0.787	0.125	1.000	0.125	0.625	0.059	0.313	0.156
$CT_{notactivated}$ (s)‡	Median	630	1,430	786	1,139	697	1,425	583	1,721	530	1,176	522	1,150
	Range	318–990	732–3,600	389–959	820–3,600	357–1,303	776–3,600	341–1,140	406–3,600	281–634	1,028–3,600	384–962	756–3,600
	P value	—	0.031	0.844	0.063	0.563	0.031	1.000	0.063	0.438	0.031	0.844	0.031
$CT_{kaolin\text{-}activated}$ (s)	Median	218	402	209	363	248	502	196	348	193	333	206	264
	Range	186–236	277–563	156–253	171–396	203–283	320–540	143–238	253–437	137–265	288–397	184–243	235–291
	P value	—	0.009	0.453	0.026	0.115	0.001	0.333	0.012	0.474	0.002	0.863	0.008
$CFT_{kaolin\text{-}activated}$ (s)	Median	60	97	55	111	63	158	64	95	55	88	58	85
	Range	49–94	83–176	44–90	77–143	52–68	75–243	56–167	68–189	39–81	63–143	36–73	55–178
	P value	—	0.003	0.378	0.002	0.729	0.013	0.356	0.093	0.535	0.049	0.418	0.128
α $angle_{kaolin\text{-}activated}$	Median	79	71	79.5	69	78	64	78	71.5	80	73	79	75
	Range	71–80	57–74	72–82	63–76	76–79	53–75	65–79	56–76	74–82	63–78	76–83	64–80
	P value	—	0.003	0.110	0.004	0.895	0.009	0.355	0.075	0.420	0.048	0.355	0.150
$MCF_{kaolin\text{-}activated}$ (mm)	Median	71.5	67.5	74	67	73	68	72	69.5	72.5	68.5	75	73
	Range	65–75	59–72	69–77	63–76	70–75	52–76	67–76	56–74	70–78	61–76	69–76	71–81
	P value	—	0.070	< 0.001	0.255	0.069	0.148	0.447	0.225	0.104	0.221	0.225	0.132

The *P* values represent differences between the value for each time point and the baseline value; values were considered significant at $P < 0.05$.

*Time immediately before dalteparin injection on days 1, 2, and 4. †Time of peak heparin activity 2 hours after dalteparin injection. ‡Samples that did not coagulate were assigned a maximum value for the assay of 3,600 seconds. CFT = Clot formation time. MCF = Maximum clot firmness.

— = Not applicable.

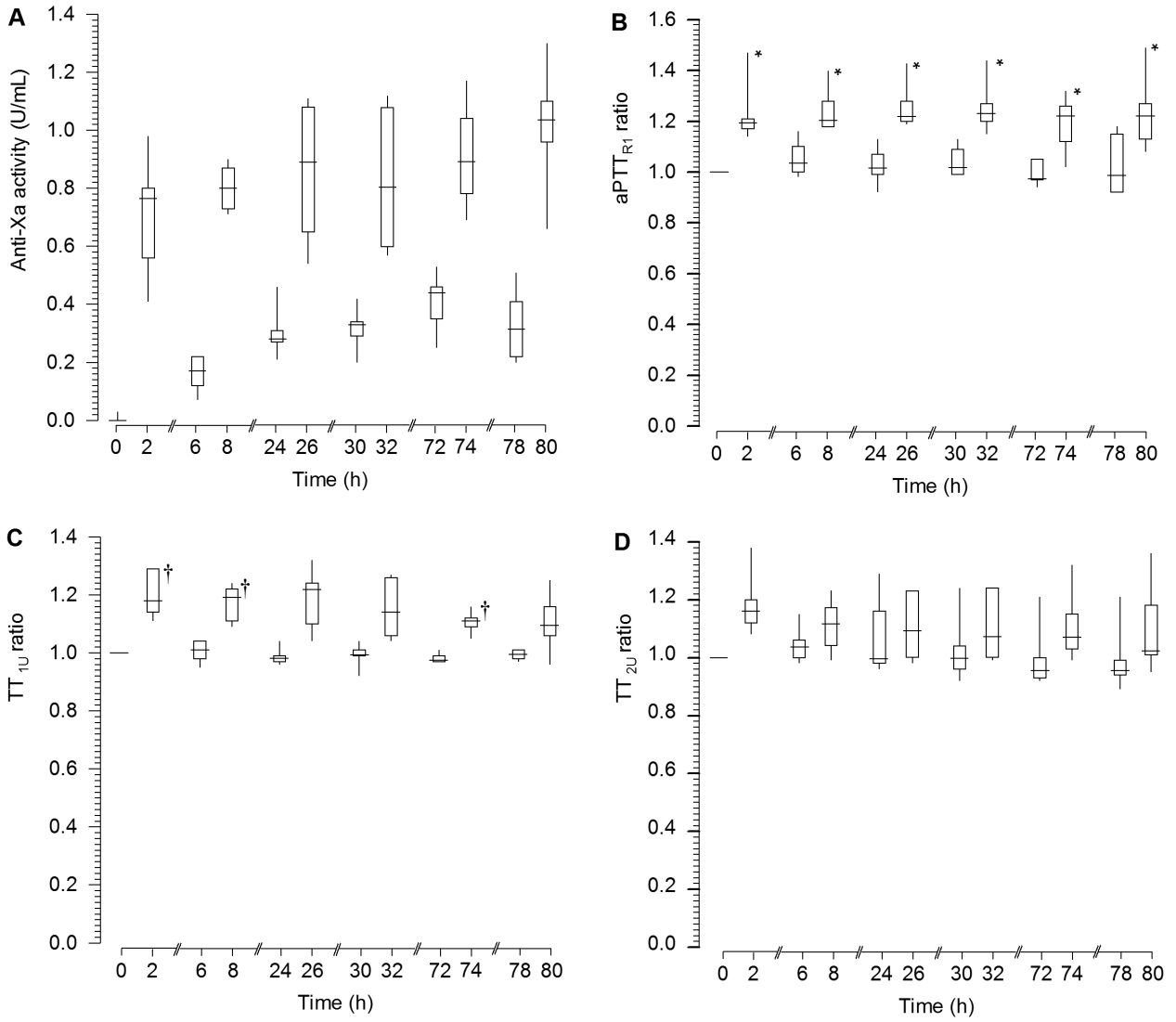

Figure 1—Box-and-whisker plots of anti-Xa activity [A] and ratios for aPTT measured with reagent 1 ($aPTT_{R1}$ [B]) and TT measured with a final thrombin concentration of 1 U/mL (TT_{1U} [C]) or 2 U/mL (TT_{2U} [D]) in samples obtained from 6 healthy cats receiving dalteparin (75 anti-Xa U/mL, SC, q 6 h for 4 days). Time of the first dalteparin injection was designated time 0 (baseline). Ratios were calculated as the value at a specific time point in relationship to the baseline value. Each box represents the 25th and 75th percentiles, the horizontal line in each box represents the median, and the whiskers represent minimum and maximum values. *†Value differs significantly (*$P < 0.01$; †$P < 0.05$) from the baseline value.

reproducible changes were found for maximum clot firmness (Table 1).

Correlation analysis

No significant correlation was found between anti-Xa activity and any of the coagulation time ratios or CT ratios ($aPTT_{R1}$, $r = 0.014$ [$P = 0.909$]; $aPTT_{R2}$, $r = 0.117$ [$P = 0.328$]; TT_{1U}, $r = -0.147$ [$P = 0.217$]; TT_{2U} ratio, $r = 0.022$ [$P = 0.857$]; CT without activation, $r = 0.148$ [$P = 0.214$]; and CT with kaolin activation, $r = -0.029$ [$P = 0.807$]).

Antithrombin activity, platelet counts, and Hct

Repeated-measures ANOVA revealed no significant effect on antithrombin activity and platelet counts, but Hct changed significantly ($P < 0.001$) over time. The Hct decreased from a baseline median value of 40.3% (range, 33.4% to 41.6%) to a median value of 31.8% (range, 27.7% to 35.9%) at 2 hours after the last dalteparin injection. A significant (paired *t* test) effect for Hct, compared with baseline Hct values, was detected beginning 6 hours after the first dalteparin injection and at all subsequent time points.

Discussion

The dosage regimen assessed in the present study was based on pharmacokinetic data for amidolytic anti-Xa activities determined in a previous study.[11] In humans, a target range of 0.5 to 1.0 anti-Xa U/mL

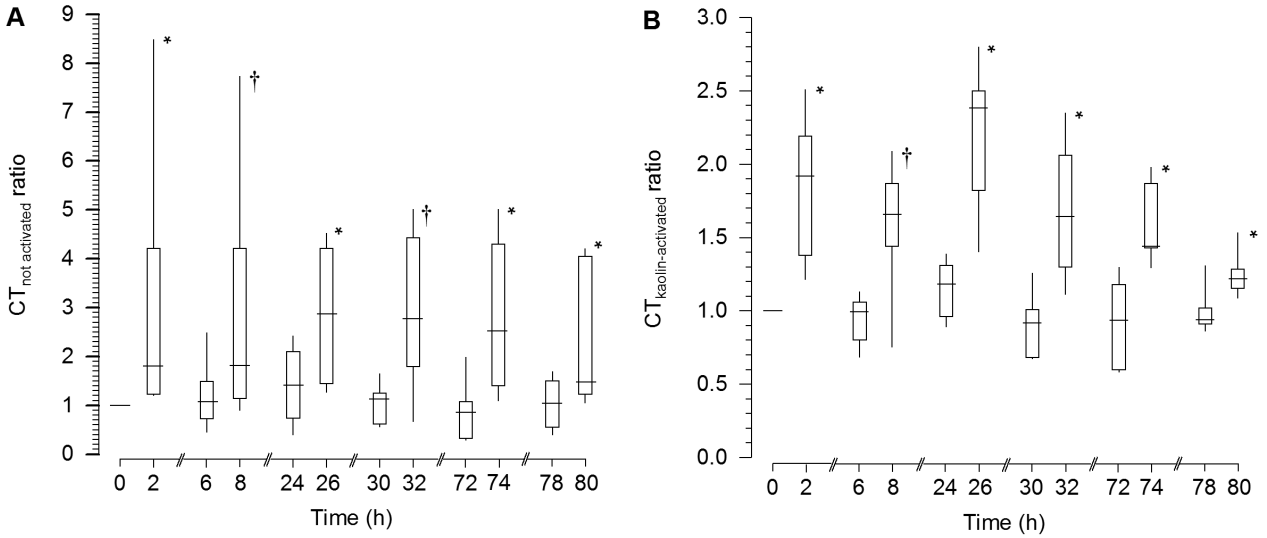

Figure 2—Box-and-whisker plots of the TEM variable CT ratio for the nonactivated analysis (A) and the kaolin-activated analysis (B) in samples obtained from 6 healthy cats receiving dalteparin. *†Value differs significantly (*$P < 0.01$; †$P < 0.05$) from the baseline value. ***See*** Figure 1 for remainder of key.

is recommended for peak values during twice-daily SC injection of LMWH for treatment of deep venous thrombosis; this target range provides an effective but safe dose for prophylactic or therapeutic use without an increased risk of hemorrhage.[4,12]

In accordance with previous studies[8,11] on dalteparin in cats, we used this target range (0.5 to 1.0 anti-Xa U/mL), although its efficacy in feline patients has not been determined. In contrast to other evaluations of dalteparin[8,10,b] and other LMWHs[9] in cats, data for the present study represented true maximal values because the experimental design took into consideration the fact that maximum plasma activities after SC injection of LMWH are reached substantially earlier in cats (approx 2 hours after dalteparin injection) than in humans.[8,11]

Median peak anti-Xa activities often were nearly at the upper limit of the target range. Therefore, beginning with the second injection, a slightly reduced dose (eg, 60 anti-Xa U/kg) may be sufficient when dalteparin is administered 4 times daily. Because of the rapid elimination of dalteparin,[11,c] a relatively short treatment interval of 6 hours was selected, which was also recommended by investigators of another study[8] who found extremely low and even nondetectable trough values for a treatment schedule of only 2 daily injections. Nevertheless, similar to results for standard treatment protocols for humans, trough values before the subsequent injection of cats in the present study regularly were below the target range. This may be acceptable because LMWHs appear to develop antithrombotic properties independent of the plasma anti-Xa activity (eg, binding to endothelial cells, releasing tissue factor pathway inhibitor from the endothelium, or expressing profibrinolytic activity).[13–15] Accordingly, investigators of another study[9] detected a limited antithrombotic effect in cats with experimentally induced venous stasis, even at time points when no anti-Xa activity was measurable. Therefore, it is possible that less frequent and thereby more practical administration of dalteparin may be sufficient for thrombosis treatment in low-risk cats, but this treatment regimen has to be assessed in clinical trials. In contrast, during the acute in-hospital phase for human patients at high risk for venous thromboembolism, trough values of > 0.5 (or 0.6) anti-Xa U/mL have been recommended.[16,17] If such trough values are also necessary for cats at high risk for thromboembolism, which should include cats with hypertrophic cardiomyopathy, an even higher frequency (> 4 times/d) for SC injections of dalteparin injections would be required. However, such a treatment regimen would be elaborate and probably could be achieved only in intensive care units.

Although statistical analysis revealed significant differences in results of certain coagulation tests, those effects were extremely minor, with a median maximum increase of 1.23 for the ratio of $aPTT_{R1}$. Even without considering the use of α adjustment for multiple comparisons, significant changes (compared with baseline values) were limited to peak times. The α adjustment was not performed because several variables did not reveal significant differences among individual time points despite a positive test result for the global comparison (eg, repeated-measures ANOVA). The limited effect on aPTT and TT was the reason that no significant correlation was found between these test results and anti-Xa activity, which is the most specific method for measuring heparin plasma activity.

These results confirmed findings of other studies that involved the administration of LMWH to cats[8,10] and humans.[13] Because of their small molecular size, LMWHs have reduced thrombin binding,[18,19] which results in a reduced effect on the common pathway of hemostasis[20] and thereby a reduced effect on conven-

tional coagulation tests.[13,21] Therefore, in contrast to monitoring patients receiving UFH treatment, conventional coagulation tests are unsuitable for use in monitoring patients receiving LMWH treatment. Although CT of TEM had a more distinct increase of the median ratio, there was also a nonsignificant correlation between results for this global screening test and anti-Xa activity. This was in accordance with results for another study[8] of cats and a study[22] of humans. However, conflicting results were reported in dogs experimentally treated with dalteparin[23] and in other studies[24,25] of humans; significant correlations between reaction time (corresponding to CT in TEM) and plasma anti-Xa activity were detected in those studies.[23–25] The present study detected significant interindividual variation in sensitivity, which may have been primarily responsible for the weak relationship. It is possible that an individually higher or lower sensitivity of the TEM assay has clinical relevance for dosage adjustment (eg, to predict hemorrhagic risk), but this has to be proven in clinical trials.

A possible influence of the decrease in Hct during the experimental period on CT and other TEM variables must be taken into account.[26,27] However, on the basis of experimental data for canine samples, these effects may have been minimal for the changes of Hct in the cats of the study reported here, whereby mean values did not decrease below the reference interval.

Kaolin-activated TEM yielded more reliable results than did nonactivated TEM, which, in part, did not have a detectable clot when anti-Xa activity was within the target range. For that reason, for the nonactivated TEM analysis, we performed statistical analysis for CT only, and the kaolin-activated method should be used for future studies.

Anti-Xa assays are considered the criterion-referenced standard for monitoring of LMWH effects in humans[13,28] and other animals,[29] but these specific tests are not widely available for routine monitoring and are rarely offered by veterinary laboratories. Monitoring after administration of LMWH is generally considered unnecessary for most patient groups in human medicine[3,12,13] because of the stable pharmacological properties of these LMWHs, which results in predictable anti-Xa activities.[30,31] Consequently, subtherapeutic or supratherapeutic blood activities with an increased risk of insufficient or adverse effects (especially hemorrhage) are observed less frequently.[32] Because the variability of anti-Xa activity in cats of the present study was low, the results indicated that these same pharmacokinetic properties can be assumed for cats. Thus, routine monitoring may be unnecessary after LMWH treatment in this species. However, routine monitoring will increase the efficacy and safety for LMWH treatment in feline patients until clinically approved treatment schedules are available.[13] Monitoring might be a necessity in certain patient groups (pregnant and obese animals or animals with renal failure), similar to the situation in humans.[3,33]

The lack of effect on antithrombin activity detected in cats of the present study is consistent with findings for humans.[34,35] Although LMWH enhances antithrombin consumption, this effect is limited in healthy subjects that have minimal quantities of activated coagulation factors in the circulation. We also did not measure an effect on platelet counts, which reflects the reduced effect of LMWH on platelets, compared with the effect of UFH on platelets.[3] In humans treated with UFH, a mild form of heparin-induced thrombocytopenia (type I) can develop during the first days of treatment, but this thrombocytopenia is unlikely to develop during treatment with LMWH.[36]

The Hct decreased significantly beginning 6 hours after the first dalteparin injection. One reason for this might have been a dilution effect caused by repeated collection of blood samples (total of approx 36 mL of blood collected during the entire study period). Another reason might have been the loss of blood through hematomas at venipuncture sites during peak anti-Xa activities, or there might have been an additional subclinical blood loss. However, this is speculative because we did not specifically examine the cats for evidence of hemorrhage (eg, blood in the body cavity). A decrease in the Hct has also been described in dogs receiving repeated injections of LMWH.[37]

A treatment regimen of 75 anti-Xa U of dalteparin/kg injected SC every 6 hours resulted in peak anti-Xa activity at the upper limit of the defined target range (0.5 to 1.0 anti-Xa U/mL). This treatment protocol or a slightly reduced dose beginning with the second injection can be recommended for clinical trials required to examine whether the anticoagulatory effects of the resulting dalteparin blood activities are adequate to effectively treat thromboembolic diseases in hypercoagulable feline patients. The low interindividual variation of peak anti-Xa activity in healthy cats, which has not yet been confirmed in feline patients, may indicate that routine monitoring for dalteparin is unnecessary in cats as well as in humans.

Footnotes

a. Defrancesco TC, Moore RR, Atkins CE, et al. Comparison of dalteparin and warfarin in the long-term management of feline arterial thromboembolism (abstr). *J Vet Intern Med* 2003;17:448–449.
b. Goodman JS, Rozanski EA, Brown D, et al. The effects of low-molecular-weight-heparin on hematologic and coagulation parameters in normal cats (abstr). *J Vet Intern Med* 1999;13:268.
c. Alwood AJ, Downend AD, Simpson SA, et al. Pharmacokinetics of dalteparin and enoxaparin in healthy cats (abstr). *J Vet Emerg Crit Care* 2005;15:1.
d. Micro-specimen cup sodium citrate, Sarstedt AG & CO, Nümbrecht, Germany.
e. Coatest heparin, Chromogenix-Instrumentation Laboratory SpA, Milano, Italy.
f. Hitachi 912, Roche Diagnostics GmbH, Mannheim, Germany.
g. Fragmin D, 2500 anti-Xa U/mL, Pharmacia GmbH, Berlin, Germany.
h. Amax Destiny Plus, Tcoag Deutschland GmbH, Lemgo, Germany.
i. CK Prest, Diagnostica Stago SAS, Asnières sur Seine Cedex, France.
j. SynthAFax, Instrumentation Laboratory Co, Lexington, Mass.

k. Test thrombin, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany.
l. ROTEM delta, Tem Innovations GmbH, Munich, Germany.
m. Star-tem, Tem Innovations GmbH, Munich, Germany.
n. Antithrombin III, universal package for analytic systems, Roche Diagnostics GmbH, Mannheim, Germany.
o. Advia 120, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany.
p. Prism, version 5.0, GraphPad Software Inc, San Diego, Calif.
q. MS Excel 2003, Microsoft Corp, Redmond, Wash.

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