

Anticoagulant effects of repeated subcutaneous injections of high doses of unfractionated heparin in healthy dogs

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Objective—To evaluate SC administration of unfractionated heparin (UFH) in accordance with a dosing regimen for high-dose treatment in dogs.

Animals—10 healthy adult Beagles.

Procedures—Two groups of dogs (5 dogs/group) were given 6 injections of heparin (500 units of UFH/kg of body weight, SC) at intervals of 8 (experiment 1) and 12 (experiment 2) hours. Blood samples were collected before and 4 hours after heparin injections to determine amidolytic heparin activity, activated partial thromboplastin time (APTT), thrombin time, antithrombin activity, platelet count, and Hct.

Results—For experiments 1 and 2, mean \pm SD heparin activities before (experiment 1, 1.32 ± 0.20 U/ml; experiment 2, 0.69 ± 0.174 U/ml) and 4 hours after the last heparin injection (experiment 1, 1.71 ± 0.30 U/ml; experiment 2, 1.10 ± 0.30 U/ml) were higher than values calculated for the regimen used in experiment 1. Results of the investigated thrombin time test system with low thrombin activity were frequently beyond the measurement range, even with UFH activities ≥ 0.6 U/ml. Moreover, a severe decrease of antithrombin activity became evident during both experiments (eg, in experiment 2 from 95.6 ± 4.8 to $59.2 \pm 6.6\%$). In each treatment group, 2 dogs developed hematomas.

Conclusions and Clinical Relevance—Calculations of the course of heparin activity after a single injection do not result in a reliable dosing regimen for high-dose heparin treatment in dogs. High-dose treatment must be monitored for each dog. Thrombin time measured with low thrombin activity is unsuitable for this purpose. (*Am J Vet Res* 2001;62:1887–1891)

The increasing importance of heparin treatment in dogs has been insufficiently represented by pharmacokinetic and pharmacodynamic studies of heparin in this species.¹ To our knowledge, there is only 1 report in which a dosing regimen for repeated SC injections has been evaluated.² Statements of that investigation are exclusively based on alterations of the screening test, activated partial thromboplastin time (APTT). When analyzed in that manner, possible species-related differences regarding the degree of APTT prolongation at defined amounts of heparin activity, however, are insufficiently considered. In addition, the influence of heparin on APTT depends on other factors, especially alterations of antithrombin activity.^{2,3}

Therefore, the purpose of the study reported here was to investigate administration of unfractionated heparin (UFH) in accordance with a dosing regimen for high-dose standard heparin treatments based on the course of heparin activity after a single injection.¹ In addition to heparin activity, APTT, thrombin time, antithrombin activity, platelet count, and Hct were measured.

Materials and Methods

Animals—Ten healthy 8- to 11-year-old Beagles especially bred for use in research were used in the study. Median age was 9 years for dogs in experiment 1 and 10 years for dogs in experiment 2. Body weight of the dogs ranged from 11 to 18 kg (median, 15 kg) for experiment 1 and 12 to 17 kg (median, 15 kg) for experiment 2. Sex distribution of dogs in experiment 1 was 1 sexually intact female, 2 castrated females, and 2 sexually intact males and in experiment 2 was 1 sexually intact female, 2 castrated females, and 2 castrated males. The project was approved by an institutional animal care and use committee (regional government of Hannover, file No. 99/153).

During the experimental period, dogs were housed separately in cages. Dogs were fed a commercially prepared food 4 hours after the start of the experiment and again 24 hours later. Dogs had ad libitum access to water. All dogs were examined at regular intervals and had been vaccinated annually against leptospirosis, infectious hepatitis, parvovirus, canine distemper, and rabies. The WBC count; plasma albumin, urea, and creatinine concentrations; and activity of alanine transaminase and glutamate dehydrogenase were measured before the start of the experiment and were found to be within respective reference ranges for all dogs.

Experimental procedure—In experiment 1, 6 injections of heparin (500 units of UFH/kg of body weight, SC) were administered to 5 healthy dogs at 8-hour intervals. This dosing regimen was calculated on the basis of antifactor-Xa activity after a single SC administration of UFH¹ to achieve a plasma heparin activity between 0.2 and 0.8 U/ml, in accordance with recommendations for humans.⁴ On the basis of results of experiment 1, a second dosing regimen was chosen empirically, and another group of 5 dogs received 6 injections of UFH (500 U/kg, SC) at 12-hour intervals (experiment 2). Dogs were injected with a commercial UFH preparation[†] from porcine mucosa with a specific activity of 5,000 U/ml. The first injection in each experiment was administered at 8:00 AM (time 0).

Collection of blood samples—During experiment 1, blood samples were collected immediately before and at the time of maximum heparin activity¹ (ie, 4 hours) after the first, second, third, and sixth heparin injections (ie, 4, 8, 12, 16, 20, 40, and 44 hours after the first heparin injection). For experiment 2, blood samples also were collected before and 4 hours after each heparin injection. Blood samples were obtained from a cephalic or saphenous vein, using sterile disposable cannulas (1.1 \times 30 mm). Blood was collected into

Received May 24, 2000.

Accepted Apr 23, 2001.

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plastic tubes containing sodium citrate (1 part of sodium citrate [0.11 mol/L]: 9 parts of blood [6 ml]) and into tubes containing EDTA (approx 1 ml). Each citrated blood sample was centrifuged immediately (2,000 × g for 20 minutes at 4 C) to prepare platelet-poor plasma (PPP). The PPP was removed and stored frozen in small aliquots at -28 C for a maximum of 30 days until analyzed.

Laboratory methods—For all samples, measurements of heparin activity, APTT with 2 different reagents, thrombin time with 2 different thrombin activities in the reagent, antithrombin activity, platelet count, and Hct were performed. Chromogenic substrate tests were used to measure heparin activity, using a factor-Xa-specific chromogenic substrate,^b and antithrombin activity,^c using a clinical chemistry analyzer^d in accordance with instrument settings furnished by the reagents' manufacturers. In the heparin assay, heparin is analyzed as a complex with antithrombin, which is included in the reagent to obtain a more constant concentration of antithrombin.⁵ The reagent also contains bovine factor Xa, which is neutralized by the heparin-antithrombin complex. The remaining amount of factor Xa is inversely proportional to the heparin content of the sample. Factor Xa hydrolyses the chromogenic substrate (S-2222), thus liberating the chromophoric group *p*-nitroaniline. The increase in absorbance (measured at 405 nm) can be used to calculate heparin activity. Standards for heparin activity measurements were prepared by diluting the heparin preparation used here with pooled plasma obtained from clinically normal dogs. Measurements of antithrombin activity were calibrated by use of the pooled canine plasma.

Coagulation assays were performed in duplicate, using a Schnitger and Gross coagulometer. The APTT was measured with 2 different reagents^{e,f} in accordance with the manufacturer's instructions. Briefly, 100 µl of PPP was mixed with 100 µl of APTT reagent and allowed to incubate (2 minutes for reagent 1,^e 3 minutes for reagent 2^f) at 37 C. This was followed by the addition of 100 µl of a solution containing 25 mmol CaCl₂/L.

Thrombin time was measured by use of a commercial thrombin time reagent^g with thrombin activities of 3 and 6 U/ml in the reagent, which are most commonly used in clinical practice. Briefly, 100 µl of PPP and 100 µl of buffer solution^h were warmed for 1 minute at 37 C, and 100 µl of thrombin solution then was added. Platelet count and PCV were determined, using a semiautomatic blood cell counter.ⁱ

Statistical analysis—Data were reported as mean ± SD or median, minimum, and maximum values, depending on

distribution of values. For global statistical comparison, 1-way ANOVA with repeated measurements for all variables with nearly normal distribution of measurement values or results of the Friedman test were calculated. In addition, a *t*-test for paired observations or the Wilcoxon test was used to compare results of various time points, particularly in comparison with values obtained for time 0. Values of *P* < 0.05 were considered significant. In addition, Holm's modification of the Bonferroni method was used for multiple comparison adjustment of the α value.

Results

For experiment 1 (SC injection of 500 units of UFH/kg at 8-hour intervals), mean ± SD plasma heparin activity was 1.66 ± 0.18 U/ml 4 hours after the third injection and nearly the same after the sixth injection (1.71 ± 0.30 U/ml; Fig 1).

Results of APTT for reagents 1 and 2 and thrombin time for 3 and 6 U/ml changed significantly (*P* < 0.001) over the course of the experiment, as determined by ANOVA or the Friedman test. From 4 hours after the first heparin injection to the end of the experiment, coagulation times of all these tests increased considerably, compared with initial values (Table 1). Four

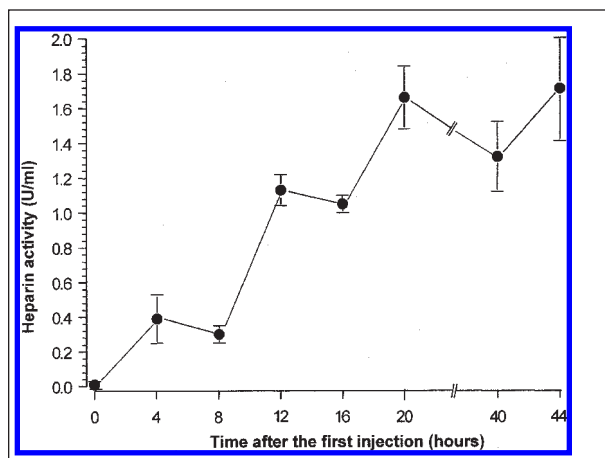


Figure 1—Plasma heparin activity (arithmetic mean ± SD) before and 4 hours after the first, second, third, and sixth of 6 injections of unfractionated heparin (500 U/kg of body weight, SC) administered to 5 healthy Beagles at 8-hour intervals.

Table 1—Activated partial thromboplastin time (APTT), thrombin time, antithrombin activity, platelet count, and hematocrit before and 4 hours after the first, second, third, and sixth of 6 injections of unfractionated heparin (500 U/kg of body weight, SC) administered at 8-hour intervals to 5 healthy Beagles

Variable	Time after the first heparin injection (h)							
	0	4	8	12	16	20	40	44
APTT (s)								
Measured with reagent 1*	15.8 (15.4–17.3)	25.0 ^(a) (18.3–27.0)	20.1 ^(a) (19.5–28.7)	46.4 ^(a) (44.8–53.6)	44.9 ^(a) (40.0–51.3)	94.4 ^(a) (64.1–423)	51.2 ^(a) (47.0–88.4)	73.6 ^(a) (57.1–166)
Measured with reagent 2†	12.0 ± 0.6	16.3 ± 1.8 ^a	15.3 ± 1.2 ^a	26.3 ± 1.8 ^b	26.2 ± 1.0 ^b	45.3 ± 8.2 ^b	33.1 ± 4.4 ^b	42.4 ± 12.1 ^a
Thrombin time(s)								
Measured with 6 U/ml *	9.6 (8.8–11.0)	16.8 ^(a) (15.8–22.5)	15.1 ^(a) (12.9–17.1)	102 ^(a) (71.5->500)	89.6 ^(a) (58.1->500)	> 500 ^(a) (NA)	> 500 ^(a) (NA)	> 500 ^(a) (NA)
Measured with 3 U/ml *	14.4 (10.5–15.8)	66.4 ^(a) (15.0–165)	39.3 ^(a) (15.9–56.1)	> 500 ^(a) (NA)	> 500 ^(a) (NA)	> 500 ^(a) (NA)	> 500 ^(a) (NA)	> 500 ^(a) (NA)
Antithrombin activity (%)†								
	104.8 ± 6.6	101.3 ± 4.0	99.0 ± 1.2	93.4 ± 2.4 ^(a)	86.2 ± 2.4 ^a	82.0 ± 1.8 ^a	62.2 ± 5.9 ^b	62.1 ± 5.8 ^b
Platelet count (X 10³ cells/µl)†								
	298 ± 40	276 ± 39	290 ± 62	283 ± 49	287 ± 40	263 ± 40	260 ± 45	244 ± 35
Hematocrit (%)†								
	48.5 ± 1.9	48.2 ± 5.6	48.7 ± 2.7	48.6 ± 3.6	46.1 ± 3.6	46.7 ± 2.3	45.8 ± 2.7	46.0 ± 3.7

*Values represent median (range). †Values represent arithmetic mean ± SD.

^{a,b}Within a row, values differ significantly (^a*P* < 0.01; ^b*P* < 0.001), compared with initial values (time 0) as determined by a paired *t*-test or the Wilcoxon test with the α value adjusted by Holm's method. ^{a,b}Within a row, *P* values were below the nominal type-I error rate (*P* < 0.05), compared with initial values (time 0) as determined by a paired *t*-test or the Wilcoxon test but above the α value adjusted by Holm's method. NA = Not applicable, because all values were greater than the limit of the test (ie, > 500 seconds).

hours after the third heparin injection, APTT for reagent 1 increased to a median value of 94.4 seconds, a 6-fold increase from the initial value (15.8 seconds). At this same time point, APTT for reagent 2 had increased 3.8 times over the initial value. Four hours after the second injection (12 hours after the first injection), thrombin time for 3 U/ml became immeasurable (median, > 500 seconds). The same applied to thrombin time for 6 U/ml in the sample obtained 4 hours after the third injection.

During the 44-hour duration of experiment 1, significant ($P < 0.001$) changes were evident for antithrombin activity, as determined by use of the ANOVA. Antithrombin activity decreased significantly ($P < 0.001$) from a mean of 104.8 ± 6.6 to $62.1 \pm 5.8\%$ (Table 1). In this experiment, platelet count and PCV did not change significantly.

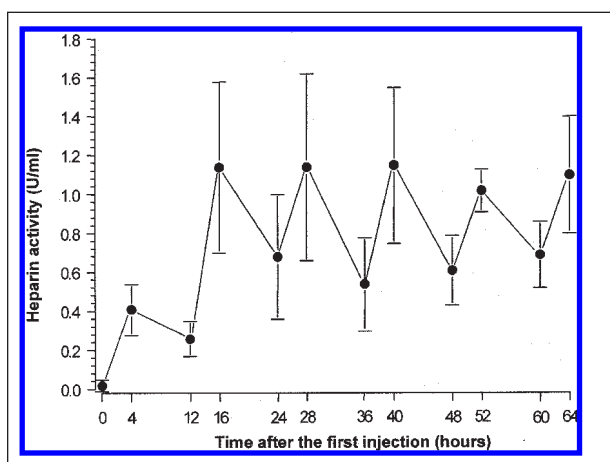


Figure 2—Plasma heparin activity (arithmetic mean \pm SD) before and 4 hours after 6 injections of unfractionated heparin (500 U/kg, SC) administered to 5 healthy Beagles at 12-hour intervals.

At the end of experiment 1, 2 dogs clearly had hematomas. One dog had hematomas around the injection sites on the lateral thoracic and abdominal walls, whereas the other dog had hematomas on the left tarsal joint.

For experiment 2, (6 injections of 500 units of UFH/kg, SC, at 12-hour intervals), mean plasma heparin activity reached nearly identical values 4 hours after the second to sixth injections (between 1.02 ± 0.11 and 1.15 ± 0.40 U/ml; Fig 2). Before the third to sixth injections, mean plasma heparin activity varied between 0.54 ± 0.24 and 0.69 ± 0.17 U/ml. Plasma heparin activity varied considerably among dogs (eg, values ranged from 0.64 to 1.60 U/ml for the 5 dogs 4 hours after the second injection).

Results of APTT for reagents 1 and 2 and thrombin time for 3 and 6 U/ml changed significantly over the course of the experiment. The APTT measured with reagents 1 and 2 and thrombin time increased significantly starting 4 hours after the first heparin injection. Four hours after the second and sixth heparin injections, APTT for reagent 1 was increased by 3.1- and 3.0-fold, respectively, compared with the initial value, whereas APTT for reagent 2 was increased 2.3-fold at the same time points (Table 2). Beginning 4 hours after the second heparin injection, thrombin time for 3 U/ml became immeasurable (median, > 500 seconds). Regarding thrombin time for 6 U/ml, only the values of the measurement 4 hours after the second and sixth heparin injections were greater than the measurement range.

During the 64-hour duration of experiment 2, significant ($P < 0.001$) changes were evident for antithrombin activity, as determined by use of the ANOVA. Antithrombin activity decreased significantly ($P < 0.001$) from a mean of 95.6 ± 4.8 to $59.2 \pm 6.6\%$ (Table 2). In experiment 2, platelet count and PCV

Table 2—Activated partial thromboplastin time (APTT), thrombin time, antithrombin activity, platelet count, and hematocrit before and 4 hours after 6 consecutive injections of unfractionated heparin (500 U/kg of body weight, SC) administered at 12-hour intervals to 5 healthy Beagles

Variable	Time after the first heparin injection (h)											
	0	4	12	16	24	28	36	40	48	52	60	64
APTT (s)												
Measured with reagent 1*	15.5 \pm 1.0	22.2 \pm 1.9 ^c	19.0 \pm 1.0 ^c	47.3 \pm 22.2 ^a	26.7 \pm 4.9 ^b	45.4 \pm 12.9 ^b	25.0 \pm 2.3 ^c	55.9 \pm 23.2 ^a	27.8 \pm 5.1 ^b	43.7 \pm 4.6 ^c	28.3 \pm 6.1 ^b	46.5 \pm 11.6 ^b
Measured with reagent 2*	11.6 \pm 0.7	15.8 \pm 1.7 ^b	13.9 \pm 0.7 ^c	26.9 \pm 8.3 ^a	18.0 \pm 2.7 ^b	26.4 \pm 5.8 ^b	17.0 \pm 1.4 ^c	29.2 \pm 7.9 ^b	18.3 \pm 2.4 ^b	25.3 \pm 2.4 ^c	18.7 \pm 3.1 ^c	26.4 \pm 4.8 ^b
Thrombin time(s)												
Measured with 6 U/ml†	9.6	14.9 ^(a)	13.0 ^(a)	> 500 ^(a)	32.3	> 500 ^(a)	24.3 ^(a)	> 500 ^(a)	15.5 ^(a)	303 ^(a)	21.3 ^(a)	> 500 ^(a)
	(9.3–10.5)	(13.5–43.3)	(11.1–15.2)	(21.4–> 500)	(12.6–38.0)	(21.7–> 500)	(12.5–49.4)	(21.5–> 500)	(14.9–40.5)	(30.2–> 500)	(15.5–36.5)	(28.0–> 500)
Measured with 3 U/ml†	14.6	73.3 ^(a)	32.5 ^(a)	> 500 ^(a)	> 500 ^(a)	> 500 ^(a)	> 500 ^(a)	> 500 ^(a)	> 500 ^(a)	> 500 ^(a)	> 500 ^(a)	> 500 ^(a)
	(13.5–16.3)	(29.4–> 500)	(19.5–51.8)	(NA)	(25.8–> 500)	(NA)	(32.1–> 500)	(NA)	(45.1–> 500)	(NA)	(47.0–> 500)	(NA)
Antithrombin activity (%)*												
	95.6 \pm 4.8	91.1 \pm 5.6	83.7 \pm 4.6 ^b	78.5 \pm 4.6 ^b	74.1 \pm 7.8 ^b	75.1 \pm 6.0 ^b	69.9 \pm 6.9 ^c	65.2 \pm 6.9 ^c	62.7 \pm 7.4 ^c	63.4 \pm 6.8 ^c	59.4 \pm 4.6 ^c	59.2 \pm 6.6 ^c
Platelet count												
($\times 10^3$ cells/ μ l)*	340 \pm 98	331 \pm 99	323 \pm 96	288 \pm 78 ^(b)	291 \pm 79 ^(a)	288 \pm 80 ^(a)	298 \pm 83 ^(b)	275 \pm 66 ^(a)	274 \pm 62 ^(a)	238 \pm 33	274 \pm 62 ^(a)	263 \pm 62 ^(a)
Hematocrit (%)*												
	48.3 \pm 4.5	47.9 \pm 2.1	47.9 \pm 2.9	44.1 \pm 4.7	43.8 \pm 3.9 ^(a)	44.2 \pm 5.2	43.4 \pm 4.9	41.6 \pm 6.5 ^(a)	41.3 \pm 4.4 ^(a)	42.0 \pm 5.7 ^(a)	40.6 \pm 5.4 ^(a)	39.1 \pm 5.2 ^(a)

*Values represent arithmetic mean \pm SD. †Values represent median (range). ^{a,b,c}Within a row, values differ significantly (^a $P < 0.05$; ^b $P = 0.01$; ^c $P < 0.001$), compared with initial values (time 0) as determined by a paired *t*-test or the Wilcoxon test with the *a* value adjusted by Holm's method. ^{(a),(b)}Within a row, *P* values were below the nominal type-I error rate (^(a) $P < 0.05$; ^(b) $P = 0.01$), compared with initial values (time 0) as determined by a paired *t*-test or the Wilcoxon test but above the *a* value adjusted by Holm's method. See Table 1 for remainder of key.

changed significantly ($P < 0.001$). Platelet count decreased significantly from a mean of $340 \pm 98 \times 10^3$ to $263 \pm 62 \times 10^3$ cells/ μ l, and Hct decreased significantly from 48.3 ± 4.5 to $39.1 \pm 5.2\%$. In this experiment, there were also 2 dogs that clearly had hematomas around the injection sites on the lateral thoracic walls (1 after the fourth injection and the other after the fifth injection).

Discussion

To our knowledge, there have not yet been any reliable values for plasma heparin activities during administration of high-dose UFH treatment in dogs. Thus, the dosing regimens reported here were calculated in accordance with recommendations for humans in which plasma heparin activities between 0.2 and 0.8 U/ml have proven to be most suitable.⁴ Surprisingly, the dosing schedule calculated from measurement values of a single administration¹ resulted in approximately 2 times higher heparin activity (1.71 ± 0.30 U/ml). Therefore, our investigation disproves the statement made by Green,⁶ who recommends 8-hour intervals of SC injections of 500 U/kg for high-dose UFH treatment in dogs because of APTT values reported for a single SC injection.

The evident accumulation of heparin can probably be attributed to elimination mechanisms for heparin. Heparin activity in blood decreases by fast-elimination zero-order kinetics and consecutive slower-elimination first-order kinetics via the kidneys.^{7,8} In phase 1 (zero-order elimination), heparin binds to endothelial cells, macrophages, and various plasma proteins (eg, histidine-rich glycoprotein, platelet factor 4, vitronectin, and von Willebrand factor) and is depolymerized into low molecular fragments such that this elimination mechanism results in saturation.

Even administration in accordance with the empirically modified dosing regimen used in experiment 2 resulted in a median heparin activity that considerably exceeded the desired range. Distinct variations of plasma heparin activity among the dogs make it even more difficult to elaborate general dosing recommendations for ill dogs, which possibly have greater changes in factors influencing heparin pharmacokinetics (eg, circulation, PCV, renal function, function of macrophages, concentration of inhibitors). This fact emphasizes the importance of the fact that high-dose heparin treatment should be specifically designed for each dog. This also became clear from severe complications attributable to bleeding observed in the study reported here, which underlines the narrow chemotherapeutic index for UFH.⁹

Heparin activity, which exceeded the desired therapeutic range in both experiments, was reflected by results of APTT and thrombin time. The degree of prolongation of these tests exceeded the recommendations for heparin treatment (APTT, 1.5 to 2.5 times initial values; thrombin time, 2 to 4 times initial values) in dogs^{2,6} and humans.¹⁰ Values of thrombin time for 3 U/ml were greater than the measurement range when plasma heparin activities were in the high therapeutic range. Therefore, analysis of results of the investigated tests reveals that, similar to humans,¹⁰ only APTT and thrombin time measured with high thrombin activity can be

used for monitoring dogs receiving high-dose heparin treatment. However, if available, a more specific chromogenic heparin activity would be preferred for this use, because only moderate correlations have been detected between results of this more specific assay and ratio values of the screening tests APTT and thrombin time.¹¹

The distinct decrease of antithrombin activity after several SC injections of heparin has been observed in dogs² and humans.¹² In 1 of those investigations,² Beagles received SC injections (200 U/kg) 4 times/d for 10 days, and antithrombin activity decreased by 39% between days 0 and 10. In the study reported here, 500 U/kg was injected SC at 8-hour intervals, and antithrombin activity decreased by the same extent after only 2 days. The decrease of antithrombin activity during heparin administration can be explained by the fact that complexes of antithrombin with activated coagulation factors are eliminated, the formation of which is catalyzed by heparin and thereby exceeds new synthesis. In this manner, the decrease of antithrombin activity in healthy animals may reflect a small degree of latent coagulation, even under physiologic conditions, that is indicated by low values for activation markers of coagulation in healthy individuals.¹³

The considerable decrease of antithrombin activity reported here documented that antithrombin has to be sufficiently supplemented during high-dose UFH treatment (eg, administration of fresh-frozen plasma). To provide satisfactory heparin effects, a minimum antithrombin activity of 70% is required in dogs,¹⁴ similar to the situation in humans. This is even more important when one considers that in patients with activated hemostasis, such as that attributable to disseminated intravascular coagulation, antithrombin activity may decrease to a much greater degree, and there often are already lower initial values.¹⁵

Decreases in platelet count and Hct that were detected in experiment 2 have to be interpreted carefully, because it was not detected in experiment 1, in which the heparin dose was even higher. Therefore, it can more likely be explained by hemodilution caused by bleeding or collection of blood samples than by possible specific effects of heparin on thrombocytes¹⁶ or erythrocytes.¹⁷

Analysis of results of the study reported here revealed that calculations of heparin activity after a single injection cannot be extrapolated to create a reliable dose regimen for high-dose heparin treatment in dogs. Analysis of results of this investigation suggests that an initial dose of 500 U/kg should be followed by reduced doses administered at 12-hour intervals in dogs. A 12-hour dosing protocol is more practical than an 8-hour protocol. It is questionable whether there can be a generally applicable dosing schedule for use in all dogs, because plasma heparin values varied considerably among our dogs despite administration of defined dosages. As a consequence, more attention should be paid to monitoring dogs receiving heparin treatment.

^aLiquemin, Hoffmann-La-Roche AG, Basel, Switzerland.

^bCoatest heparin, Chromogenix Instrumentation Laboratory SpA, Milan, Italy.

^cAntithrombin III for BM/Hitachi 704/911-systems, Roche Diagnostics GmbH, Mannheim, Germany.
^dHitachi 704 auto-analyzer, Roche Diagnostics GmbH, Mannheim, Germany.
^ePathromtin, Dade Behring Marburg GmbH, Marburg, Germany.
^fPTT-Reagent, Roche Diagnostics GmbH, Mannheim, Germany.
^gTest thrombin (30 U), Dade Behring Marburg GmbH, Marburg, Germany.
^hDiethylbarbiturate buffer solution, pH 7.6, Dade Behring Marburg GmbH, Marburg, Germany.
ⁱSysmex F-800, Sysmex Medical Electronics GmbH, Norderstedt, Germany.

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