Pharmacokinetics and pharmacodynamics of a therapeutic dose of unfractionated heparin (200 U/kg) administered subcutaneously or intravenously to healthy dogs


#: UMR 181 Experimental Physiopathology and Toxicology INRA/ENVT * Internal Medicine and **Clinical Pathology, Ecole Nationale Vétérinaire de Toulouse, 23 chemin des Capelles, 31 076 Toulouse cedex 3, France

***: Laboratoire de Recherche sur l’Hémostase et la Thrombose, CHU Purpan, 1 place du Dr Baylac, 31 059 Toulouse cedex, France

° corresponding author ; email: a.diquelou@envt.fr

Short title: Pharmacodynamics of 200 UI/kg of UFH in dogs (37 characters)

Objectives: To evaluate the effects of 200 U/kg of sodium unfractionated heparin (UFH) on coagulation times in dogs after IV and SC administration and to compare these effects with plasma heparin concentrations assessed by its anti Xa activity.

Methods: 200 U/kg of UFH were administered Intravenously (IV) and Subcutaneously (SC) to five healthy adult Beagle dogs with a wash out period of at least 3 days. Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) and plasma anti-factor Xa (aXa) activity were determined in serial blood samples.

Results: After IV injection, PT remained unchanged except for a slight increase in one dog; APTT was not measurable (> 60 s) for 45 to 90 min, then decreased regularly and returned to baseline values between 150 and 240 min. High plasma heparin concentrations were observed (C max = 4.64±1.4 aXa U/mL) and decreased according to a slightly concave-convex pattern on a semi-logarithmic curve but returned to baseline slightly more slowly (t240 to t300 min).

After SC administration, APTT was moderately prolonged (mean±SD prolongation: 1.55±0.28 x APTT t0, range [1.35-2.01]) between 1 and 4 hours after administration. Plasma anti-factor Xa
activity reached a maximum of 0.56±0.20 aXa U/mL, range: [0.42 - 0.9] after 132±26.8 min and this lasted for 102±26.8 min.

Heparin concentrations were grossly correlated to APTT; prolongation of APTT of 120 to 160% corresponded to plasma heparin concentrations range of 0.3-0.7 aXa U/mL, considered as the therapeutic range in human medicine.

**Conclusions:** As in human, pharmacokinetic of UFH in dogs is non linear. Administration of 200 U/kg of UFH SC in healthy dogs results in sustained plasma heparin concentrations in accordance with human recommendations for thrombosis treatment or prevention, without excessively increased bleeding risks. In these conditions, APTT can be used as a surrogate to assess plasma heparin concentrations. This has to be confirmed in diseased animals.

Key words: unfractionated heparin – dog – coagulation times –anti-factor Xa activity

**Introduction**

Heparin is a complex and heterogeneous glycosaminoglycan, widely used in human medicine to treat or to prevent thrombosis \(^1,2\). Its main effect is the inhibition of two major enzymes of the coagulation cascade, activated factors X (fXa) and II (fIIa), through binding to a plasma protein, Antithrombin III (AT III) *via* a specific pentasaccharide sequence \(^1\). Hypercoagulable states are far less common in veterinary practice than in human medicine. However, Disseminated Intravascular Coagulation (DIC), characterized by a hyperactivation of the hemostatic system, is recognized as a potential severe complication of various diseases in dogs, including neoplasia, septic shock or heat stroke \(^3-7\). Treatment of DIC remains a challenge, even in humans, but
heparin, Antithrombin III, activated Protein C and Tissue Factor Pathway Inhibitor have been
demonstrated to be of some benefit. Because of its low cost, heparin has also been
recommended for dogs with DIC. Various therapeutic or prophylactic protocols are available
in the veterinary literature: intravenous or subcutaneous administration, alone or with plasma
transfusion, with doses ranging from 20 to 1000 U/kg. These dose regimens are mainly
empirical or extrapolated from human clinical trials and rabbit models, and whether or not
heparin would help dogs with suspected hypercoagulation states still remains uncertain.
The potential benefits have to be balanced against the bleeding risk if overdosage occurs, especially
in dogs with DIC which already have a bleeding tendency because of the increased consumption
of coagulation factors. A better understanding of the pharmacology of heparin in dogs may help
to prevent these therapeutic hazards, but until recently, the pharmacokinetic studies of heparin in
dogs were mainly based on the interpretation of plasma coagulation times. Mischke et al in
2001 studied the effect of various doses of UFH on different screening tests of hemostasis
and their relationship with plasma aXa activity, but without pharmacodynamic analyses. Jacobs
et al in 1999 studied the pharmacokinetics of heparin using the plasma anti-factor Xa activity
to assess plasma heparin concentration, which has been shown to be more sensitive than plasma
coaulation times in assessing pharmacodynamic parameters in men.
However plasma anti-factor Xa activity determination is not a routine technique and plasma
coaulation times (mainly Activated Partial Thromboplastin Time [APTT]) are used in human
medicine to monitor the bleeding risk induced by heparin. In dogs, repeated SC
administrations of 150 U/kg unfractionated Heparin were reported by Mischke et al in 2001
to only moderately increase coagulation times (APTT and Thrombin Time) whilst having
significant plasma anti-factor Xa activity, thus demonstrating that screening tests were not suitable for UFH monitoring.

We studied the effect of the heparin dosage routinely used in the Ecole Nationale Vétérinaire de Toulouse (200 UI/kg of unfractionated heparin, SC) on plasma heparin concentration assessed by anti factor Xa activity and on plasma coagulation times in order to determine if this protocol should fulfilled the criteria of efficacy recommended in humans and could be monitored using APTT. We also studied the time-course effects of heparin administered subcutaneously and intravenously and its pharmacokinetic parameters.

MATERIAL AND METHODS

Dogs: Five healthy 3-5 year old adult Beagle dogs (4 males, 1 female), weighing 16 to 20 kg, were used in this study. No abnormalities were detected by physical, hematological or biochemical routine examinations. The dogs were allowed to drink, but food was withheld 12 hours before the beginning of each experiment.

Heparin: 200 U/kg of commercially available sodium unfractionated heparin (Héparine Choay, Sanofi Synthélabo, Gentilly, France) (5 000 U/mL) were administered:
- 1) intravenously in a single bolus through a 22G catheter (Surflo, Terumo, Cestas, France) located in the radial vein; after injection, the catheter was flushed with 1 mL of NaCl 9% to ensure that all heparin had been administered.
- 2) subcutaneously in the right thoracic wall of the dog.

Study design: Both IV and SC studies were conducted on the dogs. The sequence of administration was randomly determined and a wash-out period of at least 3 days was allowed
between the two administrations. This was considered as a suitable length of times on the basis of pharmacokinetics of heparin in man and rabbits\textsuperscript{15,17} and on previous studies on dogs\textsuperscript{11,13,14}.

Blood samples (4.5 mL) were collected from the jugular vein into vacuum tubes containing sodium citrate (3.8%, 0.5 mL) (Venoject citrate, Terumo).

For IV studies, a 0.7 mm microfuge (Venofix, Bruneau, Boulogne Billancourt, France) was placed in the right jugular vein to collect samples within the first 15 minutes following heparin administration to avoid repeated punctures. When blood was collected through this microfuge, the first milliliters of blood were discarded to avoid contamination by residual blood within the sampling device; after sampling, the microfuge was rinsed with 3 mL of NaCl 9\%.

Blood samples were centrifuged within 30 minutes after collection (3000g, 10 min at 4°C) (Sigma 3K10, Bioblock, Illkirch, France); platelet-poor plasmas were stored at -20°C until being assayed within 2 months.

Blood samples were obtained at times -20, 0, 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240 and 300 min after IV administration and at times -15, 0, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, 480, 540, 600, 660 and 720 min after SC administration.

Coagulation assays: APTT and Prothrombin Time (PT) were assessed using a steel ball semi-automated device (ST4, Stago, Asnières, France) according to the manufacturer’s specifications. For APTT determination, 100 µL of plasma were incubated at 37°C for 3 min with 100 µL of cephalin and activator (CK Prest, Stago). 100 µL of 0.025 M CaCl\textsubscript{2} were then added and the clotting time was recorded. For PT determination, 100 µL of plasma were added to 200 µL of tissue factor and calcium (Thromboplastine calcique, Biomérieux, Marcy L’Étoile, France) prewarmed to 37°C and the clotting time was recorded. The device automatically stopped if no clot appeared in 60 s. Reference intervals for APTT and PT were [10-13.5 s] and [6.5-8.5 s].
respectively. The intra and inter-assays variability were 2.9% and 2.2% respectively for APTT, and 2.1% and 1.8% for PT.

**Anti-factor Xa activity:** Anti-factor Xa activity was determined in plasma samples by a kinetic chromogenic method using a commercial kit (Stachrom Heparin, Stago) adapted to the SBA 30 Gilford automate (Ciba-Corning, Cergy-Pontoise, France) according to the specifications of the manufacturer, as described elsewhere 17. Briefly, 10 µL of plasma were added to 100 µL Antithrombin III (1 µM in Tris buffer, pH 7.4). After 3 s, 200 µL of factor Xa (5.6 nkat/mL in Tris Buffer) were added. 200 µL of factor Xa chromogenic substrate (CBS 31-39, 2.4 mM) were added after a 135 s incubation, and absorbance was recorded at 405 nm every 10 s. Calibration curves were obtained by heparin surcharge of autologous plasmas (r²=0.996). The reference interval was < 0.05 aXa U/mL.

**Data analysis:** Results are expressed as mean ± SD. Time-course parameters of HNF were determined by non compartmental analysis of anti-factor Xa activities using a computer program (Win Non Lin 4.0, Pharsight Corporation, Mountain View, CA, USA). The area under the plasma concentration-time curve (AUC) was derived using the linear trapezoidal curve extrapolated to infinity. C max were determined by visual inspection of the plasma concentration-time curves after the distribution phase. The apparent Mean Residence Time (MRT) was defined as AUMC (first moment of the plasma concentration-time profile) /AUC. The correlation between APTT and aXa activity was calculated using the Excel computer program (Microsoft France, Les Ulis, France).

**RESULTS**
**Heparin side effects**

No adverse drug reaction, bleeding tendency or hematoma was observed after heparin subcutaneous administration. After intravenous administration, one dog demonstrated a slight bleeding in the jugular vein a few minutes after heparin administration, which was easily controlled by local compression. In the other dogs, small hematomas in the jugular area could be observed at the end of the experiments.

**Subcutaneous administration:**

*Effects on plasma coagulation times*

A progressive increase of APTT started at t30 to t90 and APTT reached its maximum 90 to 150 minutes after SC administration (Fig. 1A). This increase lasted 144±25.1 min [120 - 180 min] before returning to baseline values at 420±73.5 min [300 - 480 min] after heparin administration. The APTT was increased by a ratio of 1.55±0.28 compared to the APTT initial value (range: 1.35-2.01).

No significant PT variations were observed (Fig. 1B), except for dog 5 which had a PT slightly above usual values (8.9 s) from 240 to 360 min after heparin administration.

*Plasma heparin concentration*

The evolution of plasma heparin concentration, assessed by anti-factor Xa activity was roughly similar to the APTT (Fig. 1C). A significant increase began 48±16.4 min [30-60 min] after administration and the maximum (range: 0.42 to 0.9 aXa U/mL) occurred between 150 and 240 min. The return to baseline values was observed between 420 to 480 min (mean±SD: 468±50.2 min).
**Intravenous administration:**

As complete IV administration of heparin was uncertain in dog 2, and the plasma concentration-time curve differed significantly from the data obtained in the other dogs, results obtained with this dog were not included for IV study.

**Effects on plasma coagulation times**

APTT was not measurable (APTT > 60 s) until t45 min (dog 1) to t90 min (dogs 3 and 5). It then decreased regularly and returned to baseline at 218±45 min [range: 150-240 min].

PT was almost unchanged (Fig. 2B) except in dog 5, in which increased PT (up to 10.4s) were observed for 120 minutes after administration.

**Plasma heparin concentration**

Maximal heparin concentrations (t1: 6.08±2.07 aXa U/mL) were observed in all dogs immediately after the administration (figure 2C), followed by a distribution phase of 4 minutes. Plasma heparin then decreased regularly and returned to baseline values at 240 min (dog 4) or 300 min (dogs 1 and 5). A moderate residual anti-factor Xa activity persisted at t300 in dog 3 (0.08 aXa U/mL). When plotted on a semi-logarithmic scale, anti-factor Xa activity disappeared according to a slightly concave-convex two-phase pattern (Fig. 3).

**Correlation between APTT and plasma anti-factor Xa activity**

Only APTT shorter than 60 s were taken into account. Plasma heparin concentration assessed by anti-factor Xa activity was strongly correlated with APTT, when data collected from all dogs were pooled ($r^2 = 0.89$, p<0.01) (Fig. 4) or when each dog was considered individually ($r^2$ ranging from 0.85 to 0.96, p<0.01).
Plasma heparin concentrations between 0.3 and 0.7 U/mL, i.e. considered in the therapeutic range in human medicine, corresponded approximately to a prolongation of APTT between 120 and 165 % of APTT initial values.

**Pharmacokinetic parameters of heparin after IV and SC administration**

The results are summarized in table 1. The Area Under the Curve (AUC) of the anti-factor Xa Activity was 147.9±45.7 [109.7 - 224.3] and 350.8±123.7 [270.7 - 531.7] aXa U.min.mL\(^{-1}\) for SC and IV administrations respectively (mean ±SD). The apparent Mean Residence Time (aMRT) varied from 201.4 to 250.1 min for SC study (mean ±SD: 222.2±23.7 min) and from 58.8 to 65.7 min (mean ±SD: 62.9±3.2 min) for IV study. Mean observed Cmax was 0.56±0.2 aXa U/mL after SC administration; Cmax of 4.64±1.4 aXa U/mL (mean±SD) was observed at t4min for IV study. Apparent bioavailability was 50±16%, ranging from 28% (dog 5) to 68% (dog 3).

**DISCUSSION**

This study was designed to investigate the effects of a therapeutic dose of unfractionated heparin on plasma anti-factor Xa activity, representing the plasma heparin concentration, and to determine its effects on the coagulation times routinely measured to monitor heparin therapy.

Different methods can be used to assess plasma heparin concentrations: plasma anti-factor Xa or anti-factor IIa activities, assessed by amyldolytic assays, reflect the concentration of the heparin fraction containing the pentasaccharide sequence (high AT III affinity-heparin, HAH), whereas radiolabelled heparin explores the behavior of the entire compound. Some studies have compared data calculated from anti-factor Xa and anti-factor IIa in the rabbit after IV and SC administration of UH: they were almost identical \(^{17}\). In contrast, some discrepancies between the results obtained by radiolabelled heparin and amyldolytic assay have been observed in the
rabbit\textsuperscript{18}; in our study in the dog, the decrease in plasma anti-factor Xa activity observed after IV administration was slower than was observed in dogs after a single IV injection of 1 mg/kg of $[^{35}\text{S}]$ UH (approximately 150 U/kg) \textsuperscript{20} (as much as 66\% of plasma radioactivity was lost within the first 15 min). This may be because HAH are cleared more slowly than the low affinity fraction (without the pentasaccharide) \textsuperscript{19}. Because HAH is the biologically active fraction, amydiolytic assay is the most commonly used variable to assess heparin pharmacokinetics.

After IV administration, plasma heparin disappeared according to a slightly concave-convex pattern on a semi-logarithmic plot in our study. Such concave-convex curves weren’t observed in the dog when lower UFH (25, 50 and 100 U/kg) doses were given in a previous study \textsuperscript{14}, but similar nonlinear kinetics have been observed with high doses (>100 U/kg) of UH in rabbits \textsuperscript{18,21} and humans \textsuperscript{15} and analyzed as the combination of a highly efficient, saturable heparin uptake by the endothelial cells and a linear non saturable renal elimination \textsuperscript{21-24}. In the dog, renal elimination of radiolabelled UH was observed, whereas the high relative concentrations of $[^{35}\text{S}]$ UH found in the liver and spleen were consistent with heparin uptake by the reticuloendothelial system \textsuperscript{20}. Thus, it can be hypothesized that the elimination of UH in dogs is comparable to what is observed in man and rabbits. This may be of importance when treating a dog suffering from DIC, in which the renal function may be compromised \textsuperscript{4}.

Concerning pharmacokinetic parameters, our results were consistent with previous studies \textsuperscript{14,17}. Distribution volume was close to the plasma volume (50 mL/kg) and comparable to data from other studies on the dog \textsuperscript{14} and the rabbit \textsuperscript{17}. Due to the non linear kinetics, only apparent MRT can be calculated. After SC administration, aMRT in our study (222±23 min) was quite similar to the MRT reported after 250 UI/kg SC of UH by Jacobs \textsuperscript{14} (3.7± 2.4 hours), but it was higher after IV administration of 200 U/kg than that was observed in the same study after IV
administration of 25, 50 or 100 U/kg UH in the dog \(^{14}\). However, heparin aMRT has been demonstrated to be influenced by the dose administered: the higher the dose, the longer the apparent MRT \(^{22}\).

As the APTT is influenced by heparin concentration \textit{in vivo} and \textit{ex vivo}, it has been used as a surrogate for plasma heparin concentrations. However, as has already been demonstrated in man \(^{16,25}\), the APTT lacks sensitivity when plasma heparin concentrations are very low or very high. For example, in our study, the APTT after IV administration was longer than 60 s for at least 45 min in all dogs, whereas the plasma anti-factor Xa activity varied from 10 to 2,4 aXa U/mL. As 60 s is the upper limit of the automated coagulometer, we performed manual determinations of APTT in some plasma (data not shown): no clot was observed over 600 s and the blood was considered as uncoagulable. The lack of sensitivity of APTT may also be influenced by the reagent used, as observed with different reagents in man and in dogs \(^{12,16,26}\).

However in our experiments and in the range of plasma heparin concentrations obtained after SC administration, APTT-time curves were roughly similar to those of heparin-time and APTT strongly correlated with plasma anti-factor Xa activity.

Much more convenient and far less expensive to assess than plasma anti-factor Xa, APTT is widely used in routine and it has also been correlated with the bleeding risk in men \(^2\). A 1.5 to 2.5 times prolongation in APTT \(^2\), corresponding to plasma heparin concentrations ranging from 0.3 to 0.6 U/mL \(^1\) is the target of human therapy for thrombosis and has also been recommended in DIC-treated dogs \(^5,9\). But, with our reagents, this prolongation was achieved by a heparin concentration two times higher. In this experiment, recommended heparin concentrations produced lower APTT values in dogs than in humans, as previously reported \(^{12}\).
In contrast to APTT, PT is considered insensitive to heparin, as observed in healthy men or human patients receiving heparin for thrombosis therapy. Insensitivity of PT to heparin was also observed in most dogs in this study: even after IV administration, PT was only slightly prolonged for 20 minutes, whereas APTT remained not measurable for at least 45 min. Surprisingly, one dog (dog 5) demonstrated a weak PT prolongation after SC UH administration and a stronger one after IV administration when very high plasma heparin concentrations were achieved. This might be due to an individual variation of its hemostatic system, as after SC administration, PT in this dog was slightly increased whereas it was not in dog 3, which had the highest plasma anti-factor Xa activity. Despite this isolated observation in a single dog, it can be considered that a significant increase of PT in DIC dogs treated with heparin may be due to a worsening of DIC and consumption of coagulation factors rather than to heparin therapy itself.

The dose of heparin used in this study was chosen through our experience in DIC therapy in dogs and data available in the literature. Concerning the efficiency and safety of heparin therapy protocols, SC administration of 200 UI/kg of UH induced a plasma heparin concentration in the therapeutic range between 1 to 6 hours after administration. IV bolus is not to be recommended due to the low aMRT and high peak concentrations, inducing a possible bleeding risk. A continuous infusion of UH would be interesting within a whole blood or plasma transfusion which also brings coagulation factors and ATIII. However, the UH infusion rate to maintain plasma heparin concentration in the [0.3-0.7 aXa U/mL] range is difficult to predict due to the non linear kinetic, and heparin continuous infusion protocols have been proved to increase the bleeding risks in men, compared to subcutaneous administration.
So, based on plasma heparin concentrations assessed by anti-factor Xa activity established in this study, administration of 200 U/kg of UFH SC results in plasma heparin concentrations considered as therapeutic in humans without excessively increasing bleeding risks. These concentrations corresponded to a 1.2 to 1.5 fold increase of APTT, which can be used as surrogate to plasma heparin concentration. The efficacy of such a dose regimen and the relationship between plasma heparin and APTT prolongation have yet to be confirmed in clinical trials with diseased animals.
BIBLIOGRAPHY


Legends:
Figure 1: Mean±SD Activated Partial Thromboplastine Time (APTT) (fig.1A), Prothrombin Time (PT) (fig.1B) and plasma heparin concentration assessed by anti Factor Xa activity (fig.1C) observed after subcutaneous administration of 200 U/kg of unfractionated heparin to healthy dogs.

Figure 2: Mean±SD Activated Partial Thromboplastin Time (APTT) (fig.2A), Prothrombin Time (PT) (fig.2B) and plasma heparin concentration assessed by anti Factor Xa activity (fig.2C) observed after intravenous administration of 200 U/kg of unfractionated heparin to healthy dogs.

Figure 3: Plasma heparin concentrations (mean±SD) assessed by anti-factor Xa activity (aXa U/mL) observed after intravenous administration of 200 U/kg of unfractionated heparin to healthy dogs.

Figure 4: Correlation between APTT (s) and plasma anti-factor Xa activity (U/mL) (s) after intravenous or subcutaneous administration of 200 U/kg of unfractionated heparin to healthy dogs. Plasma heparin (aXa U/mL) = 1.4165 Ln [APTT (s)]-3.485, r²=0.89.

Table 1: Pharmacokinetic parameters established after IV and SC administrations of 200 U/kg of UH in healthy dogs. AUC = area under the curve, Cmax = maximal concentration, Vd = distribution volume, aMRT = apparent mean residence time, Cl = apparent clearance, aF = apparent bioavalability.
Figure 1:
Figure 2:
Figure 3:

Figure 4:
<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>IV Results (m±SD)</th>
<th>SC Results (m±SD)</th>
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<tr>
<td>AUC (U.min.mL⁻¹)</td>
<td>350.8±123.7</td>
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<td>Cmax (U. mL⁻¹)</td>
<td>4.64±1.40 (t 4 min)</td>
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<td>Vd (mL.kg⁻¹)</td>
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<td>aMRT (min)</td>
<td>62.9±3.2</td>
<td>222.2±23.7</td>
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<tr>
<td>Cl (U.min⁻¹.kg⁻¹)</td>
<td>0.61±0.12</td>
<td>1.44±0.3</td>
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<td>aF (%)</td>
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Table 1
Figure 3 (*other possibility*): Plasma heparin concentrations assessed by anti-factor Xa activity (aXa U/mL) observed after intravenous administration of 200 U/kg of unfractionated heparin to 4 healthy dogs.