The Veterinary Journal 200 (2014) 375–381

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Enoxaparin: Pharmacokinetics and treatment schedule for cats

Reinhard Mischke [a,*,1], Jette Schönig [a,1], Elisabeth Döderlein [a], Sonja Wolken [b], Claudia Böhm [b], Manfred Kietzmann [c]

[a] Small Animal Clinic, University of Veterinary Medicine Hannover, Bünteweg 9, D-30559 Hannover, Germany
[b] Institute for Parasitology, University of Veterinary Medicine Hannover, Bünteweg 17, D-30559 Hannover, Germany
[c] Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine Hannover, Bünteweg 17, D-30559 Hannover, Germany

ARTICLE INFO

Article history:
Accepted 28 March 2014

Keywords:
Anti-FXa activity
Low molecular weight heparin
Cat
Pharmacokinetics
Treatment schedule
ROTEM
Thromboelastometry

ABSTRACT

Detailed pharmacokinetic data are not available for subcutaneously (SC) administered enoxaparin in cats and this causes difficulties in establishing treatment protocols. The aims of this study were (1) to establish pharmacokinetic data of SC administered enoxaparin and (2) to establish a treatment schedule. Six healthy cats received a single SC injection of 1 mg enoxaparin/kg and blood samples were collected before and 1, 2, 3, 4, 6, 8, 10 and 12 h after the injection. Six further healthy cats received 0.75 mg/kg every 6 h for four consecutive days and blood samples were collected before and 2 h after the first and second injection on day 1, and the first injection on days 2 and 4. Anti-factor Xa (FXa) activity, coagulation tests and thromboelastometry assays were performed.

Enoxaparin injection was well tolerated. Following the single SC injection C_{max} was 0.83 ± 0.08 anti-Xa IU/mL and in 5/6 cats was detected after 2 h (T_{max} = 110 ± 25 min). The total clearance was 23.4 ± 4.8 mL/h/kg and the terminal half-life was 2.27 ± 0.4 h. All cats receiving repeated injections reached the defined target peak range of 0.5–1.0 IU/mL by 2 h after the second injection (0.54 [0.50–0.61]; median, [minimum – maximum]) and there was no considerable accumulation subsequently. With the exception of thromboelastometry (especially non-activated), ratio values of coagulation times increased significantly although only slightly (e.g., the maximal value of median activated partial thromboplastin time ratio was 1.27). Significant, although only moderately close relationships with Spearman rank correlation coefficients between 0.424 and 0.558 were calculated between anti-FXa activities and ratios of different coagulation times.

A dosage schedule of 0.75 mg/kg four times a day seems suitable for therapeutic use of enoxaparin in cats as it leads to reproducible peak anti-FXa activities within the target range for the treatment of thrombosis in humans. The low inter-individual variation may indicate that monitoring based on anti-FXa activities is not necessary.

Introduction

In 1916 McLean first discovered heparin while he was examining canine livers during the search for pro-coagulant preparations in different tissues (McLean, 1916). Nearly 90 years later heparin is widely used for antithrombotic therapy or prophylaxis in humans and increasingly in animals. Standard (unfractionated) heparin (UFH) is a heterogenous mixture of glycosaminoglycans that form complexes with antithrombin as well as activated (a) coagulation factors (F) such as FXa resulting in their enhanced deactivation. In addition, heparin molecules bind to other plasma proteins and to cells and this leads to unpredictable pharmacokinetics (Bârzu et al., 1986; Hirsh, 1991; Young et al., 1992). UFH is therefore not suitable for out-of-hospital use (Hirsh and Raschke, 2004). To try to eliminate some of the major disadvantages of UFH, several researchers in the 1970s worked on low molecular weight heparins (LMWHs) and developed different LMWH fractions (Lockner, 1999). LMWHs are fragments of the original UFH produced by chemical or enzymatic depolymerisation and homogeneous in size (Middeldorp, 2008).

In humans and in various animal species, LMWHs have longer half-lives (Harenberg, 1990; Samama et al., 1996; Boneu, 2000) and are consequently easier to administer with prolonged time intervals of up to 1 day (Garcia et al., 2012). In addition, LMWHs show a reduced binding to cells and proteins at the injection site and after resorption into the blood circulation resulting in a high bioavailability and more predictable plasma heparin activities (Bergqvist et al., 1983; Briant et al., 1989; Hirsh and Raschke, 2004) and possibly lower bleeding complications (Weitz, 1997; Levine et al., 2004). This makes monitoring of heparin plasma activities in most human patients unnecessary (Bounameaux and De Moerloose, 2004; Hammerstingl, 2008; Garcia et al., 2012), whereas monitoring is necessary for

* Corresponding author. Tel.: +49 511 953 6200.
E-mail address: Reinhard.Mischke@tiho-hannover.de (R. Mischke).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.tvjl.2014.03.032
1090-0233/

anticoagulant treatment of thrombosis with high doses of UFH (Garcia et al., 2012). Because of their superior pharmacokinetic and pharmacodynamic properties, LMWHs have replaced UFH for many indications in human medicine (Garcia et al., 2012).

LMWH preparations have been used as anticoagulants in cats with hypertrophic and restrictive cardiomyopathies for thrombosis prophylaxis (Pouchelon et al., 1997; Smith et al., 2004) and for the treatment of associated acute thromboembolic diseases (Rodriguez and Harpster, 2002; DeFrancesco et al., 2003; Rogers et al., 2008) as well as for the treatment of thromboembolic disease associated with heartworm disease in one cat (Davidson et al., 2006). Pharmacokinetic properties of LMWHs in humans have been assumed to be valid also for cats (DeFrancesco et al., 2003; Smith et al., 2004; Rogers et al., 2008), but pharmacokinetic studies have demonstrated major differences between the species (Alwood et al., 2005; Mischke et al., 2012). Different studies that aimed to establish dosage schedules for subcutaneously (SC) administered LMWHs in cats were not able to achieve peak anti-FXa levels within the target range adopted from humans (Alwood et al., 2007; Vargo et al., 2009; Van de Wiele et al., 2010), but specific pharmacokinetic features of cats were not adequately considered.

Whereas detailed pharmacokinetic data for cats have been reported for the LMWH dalteparin (Alwood et al., 2005; Mischke et al., 2012), only limited data are available for enoxaparin (Alwood et al., 2005). Therefore, the present study aimed (1) to establish additional pharmacokinetic data for SC administered enoxaparin and (2) based on these data to calculate a dosage schedule for repeated SC administration which was then assessed in a further group of cats.

Material and methods

Study design

Single SC injection

Six clinically healthy cats received a single SC injection of enoxaparin (1 mg/kg bodyweight [BW]) and blood samples were collected before and 1, 2, 3, 4, 6, 8, 10, and 12 h after the injection.

Repeated SC injections

Six healthy cats received in total 13 SC injections of 0.75 mg enoxaparin/kg BW at intervals of 6 h on four consecutive days. Blood samples were collected before and 2 h (at the time point of maximum heparin blood plasma activity, i.e. peak activities) after the first, second, fifth (first injection on the second day) and thirteenth enoxaparin administration (first injection on the fourth day).

Laboratory tests

Blood samples were analysed for amidolytic plasma heparin (anti-FXa) activity, activated partial thromboplastin time (aPTT), thrombin time (TT), thromboelastometry using the ROTEM delta system,[1] antithrombin activity and haematological parameters (haematocrit and platelet count). Because aPTT and thrombin concentration in the TT reagent can influence sensitivity towards UFH and LMWH (Mischke and Grebe, 2000; Mischke, 2003), parallel measurements were performed with two different commercial aPTT reagents and with two different thrombin activities in the TT reagent. Based on anti-FXa activities after single SC injections, different pharmacokinetic parameters were calculated.

Animal selection

Twelve healthy Domestic Shorthair cats owned by the Institute for Parasitology, University of Veterinary Medicine, Hannover, were used for the present experiments. These animals were kept for experimental purposes in groups of 6–10 animals. The cats had not been in other experiments for at least 6 weeks. During the experiment, cats were housed in single cages with free access to water. They were fed with a commercial cat food, which was withheld 12 h before the blood samples were collected. Before enclosing animals into the experiment, health status was ascertained by thorough clinical examination and blood analysis (haematology and blood chemical profile).

The cats receiving a single injection were aged 1–9 years (median 6 years), were of different sexes (four male neutered; one female entire; one female neutered) and weighed between 2.9 and 6.0 kg (median 4.9 kg). Cats receiving repeated injections were aged 1–9 years (median 6 years), of different sexes (two male neutered; three female entires; one female neutered) and weighed between 2.9 and 5.8 kg (median 4.0 kg).

All animals in this study were used in accordance with the 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 responsible national institution (Lower Saxony State Office for Consumer Protection and Food Safety, reference number 08/1577).

Blood sample collection and preparation of sample material

Blood samples at each time point were collected directly into sample tubes from the cephalic or the femoral vein of unsedated animals using 0.9 × 40 mm (20G) needles after discarding the first blood drops to avoid contamination with tissue factor. First, two 1.3 mL plastic tubes (Sarstedt) containing one part of 0.11 mol/L (3.8%) sodium citrate to nine parts of blood were filled for measurements of anti-FXa activities, clotting times, and thromboelastometry. After that, approximately 0.5 mL of blood was collected into an EDTA sample tube for determination of haematocrit and platelet count. Immediately after collection, each sample tube was swayed gently until blood and anticoagulant had been mixed thoroughly.

Citrated blood (2 × 300 μL) was used for thromboelastometric assays with the ROTEM delta analyser. For all other haemostasis tests, the citrated blood was centrifuged at 16,000 *g* for 10 min at room temperature using a microcentrifuge. The plasma was transferred into plastic tubes and the centrifugation procedure was repeated. This platelet free plasma was then divided into small aliquots of 4 × 200 μL and frozen at –70 °C until used for analysis. Directly before analysis the plastic tubes were thawed at 37 °C in a water bath.

Heparin preparation and administration

Clexane multidose 100 mg/mL (1 mg is equivalent to 100 anti-Xa IU) (Sanofi-Aventis) containing the active substance enoxaparin-sodium (batch 01H451) was used. SC injection was performed over the lateral thorax wall using 1 mL syringes with fine graduation marks.

Laboratory methods

Anti-FXa activity

A chromogenic substrate test (Coatest Heparin, Chromogenix-Instrumentation Laboratory) was used for measurements in the auto-analyser Hitachi 912 (Roche Diagnostics) to measure anti-FXa activity. The test application was created based on manufacturers' instructions. Different dilutions of the same batch of the commercial enoxaparin preparation used in the experiment with normal cat pool plasma (prepared from identical aliquots of 25 healthy adult cats) served as standards (final concentrations: 0, 0.25, 0.5, and 1 anti-Xa IU/mL) for the calibration of the measurement.

aPTT, thrombin time

The aPTT and TT were measured with the automated coagulation analyser Amax Destiny Plus (Trinity BioTech). The test principle is based on the spherical coagulometric method. Two different aPTT reagents were used, C.K. Prest (Diagnostica Stago; former trade name PTT Reagent; reagent 1, $aPTT_{R1}$), which was reconstituted according to the test manual and SynthAFax (Instrumentation Laboratory Company; reagent 2, $aPTT_{R2}$), which was ready to use. The TT was performed with the reagent Test Thrombin (Siemens Healthcare Diagnostics) using two different final thrombin concentrations (1 IU/mL [TT_{1IU}] and 2 IU/mL [TT_{2IU}]). APTT and TT were measured according to the test instructions provided by the manufacturers of the reagents. Coagulation times were expressed as ratio values (actual values/initial values).

Thromboelastometry

Thromboelastometric assays were performed using the ROTEM delta system (TEM International). Citrated blood samples were held at room temperature for approximately 30 min before each sample was tested on two different canals, either non-activated or after specific activation of the intrinsic pathway. Both samples were recalcified with star-tem (Tem International), a system reagent for recalcifying citrated blood or plasma. One sample was activated with the activating reagent in-tem (Tem International; pipetting scheme: 20 μL star-tem, 20 μL in-tem, 300 μL of citrated blood) and the second sample remained without further activation (addition of 20 μL isotonic NaCl solution instead of the activating reagent) before starting the test cycle (3600 s). Automatic pipettes were used to minimise error of measurements. Of the automatically provided parameters by the integrated computer, only the clotting time (CT; $CT_{non\text{-}act}$ [for measurements without activation] and $CT_{in\text{-}tem}$ [for measurements with activation using the in-tem reagent]) were used in the present study. CTs were censored at 3600 s if there was no clot detected until this time point. Results of CT are reported as ratio values (actual values/initial values).

[1] See: http://rotem.de/site/.