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Anticoagulant activity of oral rivaroxaban in healthy dogs

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ABSTRACT

Rivaroxaban is an oral, direct factor Xa inhibitor used in human thrombotic disorders. In view of the in vitro concentration dependent anticoagulant effects of rivaroxaban in dogs, the time course of its anticoagulant effects was characterized in healthy dogs. Twenty-four healthy Beagles were randomized into three groups ($n = 8$ per group) and received orally either a placebo or 20 mg rivaroxaban once or twice at an 8 h interval. Fifteen blood samples were collected over a 30 h period, and blindly assayed for prothrombin time (PT), activated partial thromboplastin time (aPTT), tissue factor induced thrombin generation (TG) and anti-factor Xa activity. Thromboelastography (TEG) was evaluated at 0, 1, 4, 8 and 24 h. Peak/baseline anticoagulant effect ratios were analyzed with generalized linear models using β distributions and times to return to baseline with survival analyses ($\alpha = 0.05$). Peak/baseline anticoagulant effect ratios of PT, aPTT, anti-factor Xa activity, TG and R (TEG) differed significantly between placebo and both rivaroxaban groups ($P < 0.0001$). The peak anticoagulant effect of rivaroxaban occurred 1.5 to 2 h after dosing. The median return to baseline occurred significantly sooner ($P < 0.01$) with 20 mg rivaroxaban administered once (7.9–18.7 h) versus twice (17.5–26.8 h). The inter-individual variability differed amongst assays, but overall was moderate to large. No adverse effects were recorded. Twice oral administration of 2 mg/kg rivaroxaban at an 8 h interval maintained 24 h anticoagulant activity, but larger studies are needed to establish guidelines for the use of rivaroxaban in dogs.

Introduction

In dogs, thrombosis is a major complication of many acquired conditions (Heidi and Byers, 2001; Kristensen et al., 2008; Laurenson et al., 2010; Kavanagh et al., 2011; de Laforcade, 2012; Kittrell and Berkwitt, 2012a; Lake-Bakaar et al., 2012; Respess et al., 2012). Anticoagulants commonly used in canine practice present variable effects from patient to patient, and bleeding risks are a concern (Kittrell and Berkwitt, 2012b; Smith, 2012). Moreover, some owners are reluctant to give subcutaneous injections (Eymin and Jaffer, 2012).

Rivaroxaban is a specific factor Xa inhibitor approved by the USA Food and Drug Administration (FDA) in 2011 for the prevention and treatment of thrombosis in human beings (Turpie et al., 2012). The cell-based description of the coagulation model shows the pivotal role of factor Xa in both the initiation and propagation phases (McMichael, 2012). Rivaroxaban acts on free factor Xa, as well as prothrombinase complex and clot bound factor Xa, making it a powerful anticoagulant (Weitz, 2011).

Roehrig et al. (2005) have described the binding site of rivaroxaban on human factor Xa. Alignment of the human and canine activated factor Xa heavy chains reveals 202/233 (87%) identities, 222/233 (95%) positive substitutions, and 0/233 gaps in their respective amino acid sequences. Moreover, 13/14 (93%) of the human amino acids interacting with rivaroxaban are identical to the canine isoform, which makes this drug potentially effective in dogs. Only one amino acid that does not directly interact with rivaroxaban differs between both binding sites, representing substitution by another basic amino acid (Lys96Arg).

An in vitro study confirmed the anticoagulant activity of rivaroxaban on canine plasma; anti-factor Xa activity and thrombin generation (TG) were the most sensitive parameters for detection of anticoagulation effects (Conversy et al., 2013). The same parameters were found in human medicine, although monitoring is not considered to be essential because of the predictable pharmacokinetic and pharmacodynamic profile of rivaroxaban (Samama et al., 2010; Harenberg et al., 2011; Douxfils et al., 2012).

The literature contains limited data on the clinical use of rivaroxaban in dogs. A pharmacokinetic study showed that rivaroxaban was absorbed in dogs with 60–86% average bioavailability and that its plasma concentrations increased linearly with

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increasing dose (Weinz et al., 2005). In addition to being excreted, rivaroxaban can be converted into 18 inactive metabolites through biotransformation pathways that minimally differ across humans, rats and dogs (Lang et al., 2009).

To our knowledge, no published in vivo study has evaluated the pharmacodynamics of rivaroxaban in dogs. We hypothesized that oral rivaroxaban would provide a significant anticoagulant effect in healthy dogs. The aims of the study were to: (1) characterize the time-course of the pharmacological effects of rivaroxaban in healthy dogs after oral administration; (2) determine the duration of the anticoagulation; (3) characterize the inter-individual variability; and (4) determine the most sensitive assay to evaluate rivaroxaban-induced anticoagulation.

Materials and methods

Experimental design and determination of oral rivaroxaban dosage

Twenty-four healthy Beagles from the institutional animal colony were enrolled (16 neutered females, eight intact females; 1.5–6.5 years of age; 7.5–13.1 kg body weight). Health status was determined by physical examination, complete blood count (Advia 120, Bayer HealthCare), serum biochemistry (Unicel DxC 600, Beckman Coulter) and urine specific gravity. Exclusion criteria were estrus, thrombotic and/or hemorrhagic events, manipulations or coagulation altering medications given in the month prior to the study. The study protocol was approved by the institutional bio-ethics committee (approval number 12-Rech-1649; date of approval 12 March 2012).

To determine the first-in-dog oral therapeutic dose of rivaroxaban, we assumed that concentrations of rivaroxaban identical to those in human beings would yield identical anticoagulant effects, implying that pharmacokinetics are the only source of interspecies variability in therapeutic efficacy (Toutain and Bousquet-Mélou, 2004):

$$\text{Dose}_{\text{new species}} = \text{Dose}_{\text{reference species}} \times \text{Clearance}_{\text{new species}} / \text{Clearance}_{\text{reference species}}$$

Assuming a 60 kg average weight for human beings, and substituting the human therapeutic dose and apparent total clearance of the drug from plasma after oral administration (CL/F) values of rivaroxaban reported by Mueck et al. (2013), together with the canine CL/F values reported by Weinz et al. (2005) at oral doses of 1 and 3 mg/kg, the above equation returned values of 2.2 mg/kg and 2.9 mg/kg, respectively, which we rounded to the nearest integer value. Therefore, the chosen test dosages were 2 mg/kg once and twice daily. Considering the standard weight of the Beagles from the colony, it was decided to administer a 20 mg tablet of rivaroxaban to each dog at each time point, corresponding to an approximate oral dose of 2 mg/kg.

Dogs were randomized evenly across three treatment groups and monitored for 30 h: (1) control: placebo pill (100 mg lactose tablets, Odan Laboratories) PO at 0 and 8 h; (2) a group receiving 20 mg rivaroxaban (Xarelto, 20 mg tablets, Bayer HealthCare) PO at 0 h; and (3) a group receiving 20 mg rivaroxaban (Xarelto, 20 mg tablets, Bayer HealthCare) PO at 0 and 8 h.

Twenty-four hours prior to entry in the study, a central venous catheter (ES-04306, Arrow International) was placed in all dogs under sedation with 3 μg/kg IV dexmedetomidine (Dexdomitor, Pfizer Animal Health), then reversed with 0.03 mg/kg IM atipamezole (Antisedan, Pfizer Animal Health). Catheters were flushed with heparin-free physiological saline during the study. A dry, low fat diet (Gastrointestinal Low Fat Veterinary Diet, Royal Canin) was offered 12 h before and 15 h after the beginning of the study. All dogs remained under veterinary supervision in the same environment, with free access to water, during the course of the study.

Coagulation assays

Citrated blood (4.5 mL) was collected into a buffered sodium citrate (9NC) sterile blood collection tube (Becton-Dickinson) from the central venous catheter at 0, 0.1, 0.33, 0.66, 1, 1.5, 2, 4, 6, 8, 8.5, 9, 11, 15, 24 and 30 h after administration of rivaroxaban. Prior to each sampling, 5 mL of blood was set aside and reinjected afterwards to avoid hemodilution of the tested samples. Platelet poor plasma was harvested from the sample by double centrifugation (Centrifuge 5810 R, Eppendorf) at 2532 *g* for 10 min at room temperature, separated into 1.5 mL aliquots in plastic tubes (Screw cap Micro Tube 2 mL, Sarstedt Aktiengesellschaft) and stored at −80 °C pending analysis for prothrombin time (PT), activated partial thromboplastin time (aPTT), anti-factor Xa activity and thrombin generation (TG). Samples collected at 0, 1, 4, 8 and 24 h were

Table 1
Medians (range) of coagulation assay values at baseline and peak in dogs treated with two different doses of rivaroxaban compared to control dogs.

				Thrombin generation				
	PT (s)	aPTT (s)	OD (anti-X activity)	Lag (min)	Peak (nM)	TTpeak (min)	ETP (nM.min)	Rate index (nM/min)
Control dogs								
Baseline value	7.4	11.6	0.68	1.3	119.9	3.2	333.0	59.9
Median (range)	(6.6–7.5)	(10.1–13.0)	(0.66–0.73)	(1.2–1.3)	(95.8–123.6)	(3.0–4.2)	(307.0–403.0)	(31.8–74.0)
Peak change value	7.6	11.9	0.66	1.3	108.0	3.3	309.7	55.1
Median (range)	(6.9–8.0)	(10.7–13.6)	(0.64–0.73)	(1.3–1.7)	(95.8–115.4)	(3.0–4.2)	(268.0–371.0)	(31.8–71.8)
Time to peak	0.7 h	1.25 h	8.3 h	0.1 h	0.3	0.2	0.7	0.2
Median (range)	(0.2–8.5 h)	(0.1–6.0 h)	(1.0–15.0 h)	(0–1.5 h)	(0–2.0)	(0–0.7)	(0.1–2.0)	(0–0.7)
2 mg/kg once daily								
Baseline value	7.1	11.3	0.68	1.3	114.1	3.3	330.5	57.1
Median (range)	(6.9–7.5)	(10.8–13.1)	(0.64–0.72)	(1.0–1.7)	(82.4–124.8)	(2.7–3.9)	(268.3–378.3)	(35.4–86.1)
Peak change value	9.1	14.2	0.06	3.4	19.4	8.6	133.3	3.7
Median (range)	(8.1–15.3)	(12.7–20.2)	(0.01–0.22)	(3.0–4.0)	(11.4–38.9)	(7.2–10.6)	(78.7–217.7)	(2.0–9.2)
Time to peak	1.5 h	1.3 h	1.5 h	0.8	1.5	1.1	1.8	1.5
Median (range)	(0.7–4.0 h)	(0.67–4.0 h)	(0.7–4.0 h)	(0.7–2.0)	(0.7–4.0)	(0.7–4.0)	(0.7–4.0)	(0.7–4.0)
2 mg/kg twice daily								
Baseline value	7.2	11.6	0.7	1.3	120.3	3.1	337.7	65.5
Median (range)	(6.9–7.5)	(11.2–14.3)	(0.6–0.7)	(1.2–1.6)	(103.7–136.6)	(2.8–3.8)	(288.3–431.6)	(44.7–79.4)
Peak change value [a]	10.2	15.4	0.04	3.7	15.1	8.9	113.7	3.0
Median (range)	(8.1–13.1)	(13.3–17.2)	(0.02–0.08)	(3.0–4.6)	(12.1–30.6)	(7.0–10.1)	(86.3–164.3)	(2.1–7.7)
Time to peak [a]	1.5 h	2.0 h	2.0 h	1.5 h	1.3	1.8	1.3	1.5
Median (range)	(1.0–2.0 h)	(1.0–2.0 h)	(1.5–4.0 h)	(0.7–2.0 h)	(0.7–2.0)	(1.0–4.0)	(0.7–2.0)	(0.7–2.0)

[a] Given that two peaks were observed with twice daily administration, only the highest or lowest of the 2 peaks are presented.
PT, prothrombin time; aPTT, activated partial thromboplastin time; OD, optical density; ETP, endogenous thrombin potential; Tmax, time of maximum absolute difference with baseline.