The Evaluation of Rivaroxaban: A New Oral Anticoagulant in Cats

by

AMY DIXON-JIMENEZ

(Under the Direction of Benjamin M. Brainard)

ABSTRACT

Up to 41% of cats diagnosed with hypertrophic cardiomyopathy will develop

systemic arterial thromboembolism (ATE). ATE is a devastating sequela since only 37%

to 45% of cats survive their first thromboembolic event. Currently available treatments

include the use of antiplatelet agents such as aspirin and/or clopidogrel or anticoagulant

medications such as unfractionated or low-molecular weight heparins. However, these

treatments have not been shown to prevent primary ATE and clopidogrel is associated

with a 49% recurrent event rate. Rivaroxaban is an orally administered direct inhibitor of

activated Factor X that is approved for prophylaxis of deep vein thrombosis, pulmonary

embolism, and for the prevention of stroke in patients with non-valvular atrial fibrillation

and acute coronary syndrome. The primary goal of this study was to evaluate the

pharmacokinetic and pharmacodynamic profile of single and multiple oral doses of RVX

in healthy adult cats.

INDEX WORDS: coagulation, cardiomyopathy, thromboembolism, hemostasis

THE EVALUATION OF RIVAROXABAN: A NEW ORAL ANTICOAGULANT IN

CATS

by

AMY DIXON-JIMENEZ

B.A., Boston College, 1996

D.V.M., Purdue University, 2002

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial

Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2016

THE EVALUATION OF RIVAROXABAN: A NEW ORAL ANTICOAGULANT IN

CATS

by

AMY DIXON-JIMENEZ

Major Professor: Benjamin M. Brainard
Committee: Gregg S. Rapoport
                     Steven D. Holladay

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2016

DEDICATION

This thesis is dedicated to my husband, David Jimenez, who supported me throughout my graduate program and residency. It is also dedicated to my dogs, both past and present (Texas, Dexter and Drogo), who kept me company during my writing and without them this manuscript would not be complete.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Coagulation abnormalities are commonly encountered in small animal practice. Diseases associated with hypercoagulability and thrombosis in veterinary medicine include immune-mediated hemolytic anemia, protein losing enteropathies, protein losing nephropathies, neoplasia, systemic inflammation, hyperadrenocorticism, diabetes mellitus, hypothyroidism, heartworm disease, and cardiac disease[1-3]. The most effective anticoagulant medication and dosing regimen for use in patients with these diseases has yet to be elucidated through controlled, prospective studies. In addition, the tests of choice for the monitoring of anticoagulant therapy along with the pharmacodynamic targets for thromboprophylaxis have yet to be defined. With new anticoagulant drugs on the horizon, the treatment and prophylaxis of thrombosis may become more tailored to the veterinary species.

The oral, coagulation factor Xa (FXa) inhibitor, rivaroxaban has been approved for use in people to prevent arterial and venous thromboembolism.[4] In veterinary patients, cats with hypertrophic cardiomyopathy (HCM) or other significant cardiac disease are considered to be at high risk for developing systemic arterial thromboembolism (ATE). In fact, the occurrence of ATE in cats with cardiomyopathy ranges from 13-17%,[5,6] and 41-48% of cats have gross or histopathologic evidence of thromboembolism at necropsy.[7-9] While anticoagulants are frequently used in veterinary medicine, they are not

ideal for long-term therapy, either due to the need for frequent injections (unfractionated heparin, low-molecular weight heparin), due to difficulty with accurate oral dosing (warfarin), or due to safety concerns for bleeding events (warfarin). There is thus a need for a safe, predictable, oral anticoagulant for cats with underlying cardiac disease that are predisposed to arterial thromboembolism. RVX holds promise because it may be orally administered once to twice daily without extensive monitoring, and is generally not affected by diet or other variables. This study was designed to test the safety and anticoagulant efficacy of rivaroxaban in healthy cats. The following literature review will cover the physiology of hemostasis and the balance between hemorrhage and thrombosis. Next, the veterinary indications for anticoagulant use will be covered. This is followed by a review of feline ATE including pathophysiology, clinical signs, treatment options and prognosis. The last sections of the literature review will focus upon currently available anticoagulant medications, antiplatelet agents, and thrombolytic agents. The mechanism of action, indications for people, and results of studies conducted in veterinary medicine are discussed. After this review, our studies will be discussed in chronological order. Our goals were to (1) describe the *in vitro* efficacy of rivaroxaban in feline whole blood (2) describe the *in vivo* efficacy of rivaroxaban given intravenously (IV) and (3) describe the pharmacokinetic and pharmacodynamic effects of multiple RVX doses given orally for one, three, and seven days. Further evaluation of RVX occurred with a long-term (30 day) study of the drug dose that correlated with optimal anti-Xa activity and prolongation of coagulation parameters.

CHAPTER 2

ANTICOAGULANTS[a]

Physiology of Hemostasis

Hemostasis is a tightly regulated and precisely balanced interaction between various plasma proteins, blood cells, and the vascular endothelium. This regulation allows localized coagulation at sites of vascular injury while preventing systemic thrombosis. When regulation of coagulation is disturbed, excessive bleeding or abnormal thrombus formation may result. The diagnosis and management of thrombotic disease is important in human and veterinary medicine due to the associated morbidity and mortality. There are up to 600,000 diagnosed cases of human venous thromboembolism (VTE) each year, primarily manifested as deep vein thrombosis and pulmonary thromboembolism.[10] People considered at risk for thrombosis include those with inherited disorders (such as protein C deficiency or the factor V Leiden mutation), cancer, heart disease, atrial fibrillation, and acute coronary syndrome, in addition to victims of trauma, and patients undergoing total hip replacement, total knee replacement, major orthopedic surgery, prolonged immobilization, and those receiving certain drugs (e.g. oral contraceptives).[10-13] The risk of venous thromboembolism is reported to be as high as 50-80% for patients that have knee or hip replacement.[14] Moreover, patients with atrial fibrillation have an increased risk of thromboembolic stroke by up to five-fold compared to people with normal heart rhythm.[15] In veterinary medicine, disorders of hemostasis are frequently encountered in small animal practice and in the critical care setting. Animals are generally thought to acquire a hypercoagulable state secondary to cardiac, metabolic, infectious, and inflammatory diseases.[16]

In health, an intact vascular endothelium prevents thrombosis from occurring through several key mechanisms. The endothelial cell is itself antithrombotic and inhibits platelet attachment through its negatively charged surface. Tissue factor pathway inhibitor (TFPI) is localized to the endothelial cell surface, where it decreases the pro-coagulant interaction of tissue factor (TF) with factor VIIa (FVIIa), an important initiator of *in vivo* clot formation. In addition, endothelial cells actively secrete vasodilators and substances that decrease or inhibit platelet activation. These substances include prostacyclin (PGI2), ecto-adenosine diphosphatase (ADP-ase) and nitric oxide.[17] The glycocalyx coats the luminal surface of vascular endothelial cells. In addition to selectins and other signaling molecules, the glycocalyx anchors heparan sulfate, a glycosaminoglycan, which promotes the anticoagulant activity of antithrombin (AT).[11] The presence of heparan sulfate markedly enhances AT inhibition of thrombin, Factor Xa (FXa) and Factor IXa (FIXa).[11,18] Protein C is also an endogenous inhibitor of coagulation. Activated protein C (aPC) is generated when thrombin binds thrombomodulin (TM) on the endothelial surface. Activated protein C, along with the cofactor protein S, inactivates coagulation factors Va (FVa) and VIIIa (FVIIIa), and also binds thrombin, thereby decreasing fibrin formation.[2] Vascular endothelial cells also create a physical barrier that prevents pro-thrombotic substances such as collagen, fibroblasts, and TF from contacting the cellular and plasma proteins within the circulation.[11]

Plasma proteins that participate in coagulation must be activated from their inactive circulating form. Most of the soluble coagulation factors are serine proteases that become sequentially activated, ultimately resulting in the formation of thrombin from

prothrombin. Traditionally, the plasma coagulation components have been described by the cascade model, which describes a series of progressive factor activation steps divided into the extrinsic, intrinsic, and common pathways. The extrinsic (or TF) pathway initiates coagulation though the interaction of TF and FVIIa.[19] Clinically, the prothrombin time (PT) is used to evaluate the factors that make up the extrinsic pathway and the common (shared) pathway. The intrinsic pathway (also known as the contact activation pathway) initiates coagulation through interaction with negatively charged surfaces (such as might be found on indwelling venous catheters) causing activation of factor XII (FXIIa) within the vascular system and the sequential activation of factors XI, IX and VIII (FXIa, FIXa, FVIIIa). This pathway also requires the zymogen prekallikrein and the co-factor high molecular weight kininogen (HMWK), which has a crucial binding site for charged surfaces.[19] The activated partial thromboplastin time (aPTT) is the screening test used clinically to evaluate the components of the intrinsic pathway and the common (shared) pathway. The intrinsic and extrinsic pathways converge onto the common pathway, which describes the sequential activation of factors X and V (FXa, FVa), which together promote thrombin formation from prothrombin (factor IIa) and ultimately, fibrin from fibrinogen (FIa).[20]

Despite the convenience of the cascade model, recent evidence suggests that the majority of *in vivo* coagulation is initiated via the extrinsic pathway.[21] The cell-based model of coagulation integrates current understanding of the interaction between the extrinsic, intrinsic, and common pathways, and describes three stages of clot formation: initiation, amplification and propagation. This model emphasizes the role of TF for initiation of coagulation, and the need for a pro-coagulant surface to support clot

formation. The surface of an activated platelet frequently provides this pro-coagulant surface. Cells that express TF are typically located in extravascular locations, however circulating cells (monocytes, neoplastic cells, and microparticles released from endothelial cells, platelets, or monocytes) can also express TF and can activate coagulation in the intravascular space. A small concentration of FVIIa freely circulates in the vascular space and, once exposed to a TF-bearing cell, quickly forms the TF-FVIIa complex. This complex catalyzes the conversion of more FVII to FVIIa, which is then available to bind more exposed TF.[20] Complexed FVIIa-TF supports the generation of small amounts of FVa, FIXa, and FXa.[20] Small amounts of thrombin are then formed through the action of FXa-FVa. Thrombin and FIXa then diffuse a short distance from the TF bearing cell to the surface of nearby platelets where the amplification stage begins.

The binding of thrombin to the platelet surface causes a reorganization of surface phospholipids, in addition to platelet shape change, and the release of platelet granules, which contain numerous procoagulant factors.[20] Thrombin also serves to promote the production of more FXIa and FVa. Importantly, thrombin also helps to separate the components of the circulating vWF-FVIII complex so both are free to participate in coagulation. vWF is critical for platelet localization, adhesion and aggregation at sites of vascular injury, and FVIII acts as a cofactor for FIX and for the activation of FX.[20]

Propagation occurs directly on the platelet surface. FVIIIa complexed with FIXa form the tenase complex, which produces large amounts of FXa.[20] FXa then interacts with FVa (forming the prothrombinase complex) to produce thrombin through the cleavage of prothrombin. If not inactivated, thrombin drives the formation of fibrin from

fibrinogen and also provides positive feedback for the formation of FXIa and tenase complexes. The individual fibrin molecules are then cross-linked by FXIIIa and form a meshwork to seal over the site of injury.

Thrombus formation is promoted when there is endothelial damage, systemic hypercoagulability and/or alterations of blood flow (including stasis). These determinants were first described by Virchow in 1856, and describe alterations that can occur within the arterial or venous system.[22] The amount of platelets and fibrin contained within a thrombus or thromboembolus varies based upon its origin in the vasculature. Arterial clots are generally formed under conditions of high shear and include platelets held together by fibrin strands (also known as "white clots").[23] Clots formed within the venous system and under low shear conditions consist mostly of fibrin and red blood cells ("red clots").[23] Due to these characteristic distinctions, therapies to prevent arterial thrombus formation have traditionally included medications that decrease platelet function. Anticoagulant medications directly impact the plasma coagulation system and are classically used to inhibit or prevent growth of venous thrombi in human patients. The use of anticoagulants over antiplatelet agents is preferred in people when the mechanism of thrombus formation is blood flow stasis, as in atrial fibrillation and deep vein thrombosis. Clinically, the use of anticoagulants for arterial thromboembolism is beneficial and confers a decrease in the risk of ischemic stroke by 40-50%.[24] A meta-analysis of 29 studies evaluated the use of anticoagulants versus antiplatelet drugs for stroke prevention in patients with atrial fibrillation. It was found that the risk of thromboembolism was reduced by 64% in patients receiving warfarin compared to 22% for those receiving aspirin.[24] Combination therapy consisting of anticoagulant and

antiplatelet drugs may be used in order to target clotting through the inhibition of coagulation factors and prevention of platelet initiation, amplification and propagation. Currently, therapy using both antiplatelet and anticoagulant drugs is frequently employed in people in order to maximize efficacy, regardless of venous or arterial thrombus origin or the mechanism of thrombus formation.

The process of fibrinolysis is an important part of hemostasis. In order to restore blood flow in a region where a thrombus has occluded blood flow, fibrinolysis must occur. An ample volume of fibrinolytic inhibitors normally keeps this system in an inactive state and fibrin is commonly required as a co-factor to activate the fibrinolytic system.[19] Plasminogen is integral to the process of clot dissolution, and plasminogen molecules are integrated into the forming clot to exist within the structure. Plasminogen is synthesized in the liver and has a long-circulating half life of 2 days.[19] Mammalian plasminogen activators, tissue-type plasminogen activator (tPA) and urokinase plasminogen activator (uPA), transform plasminogen into plasmin, initiating hydrolysis of fibrin, fibrinogen, and activated factors Va, VIIIa, IXa, XIa, and XIIa. The binding of plasminogen to lysine residues on the terminal portion of the fibrin molecule enhances plasminogen activation. Further plasminogen activation occurs in the presence of partially degraded fibrin, which has even more lysine sites available for binding.

Endothelial cells secrete tPA in response to a number of stimuli including bradykinin, acetylcholine, α-adrenergic agents, histamine and platelet activating factor (PAF).[19] The activity of secreted tPA is low in the absence of fibrin. In the presence of fibrin, tPA binds clot-bound fibrin with relative specificity and activates fibrin-bound plasminogen. Additionally, circulating plasminogen may be activated by tPA, but to a

lesser degree than with clot-bound plasminogen. The plasmin cleaved from clot-bound and circulating plasminogen degrades fibrinogen and fibrin molecules. Similar to tPA, uPA is a plasminogen activator. However, unlike tPA, plasminogen activation with uPA does not require fibrin.[19] Plasminogen activation within the vasculature is primarily achieved through tPA activity while plasminogen activation within the extravascular space is primarily achieved by uPA.

Inhibitors of fibrinolysis create a balance between thrombus formation and dissolution of clots. The major fibrinolytic inhibitors include plasminogen activator inhibitor-1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI), and α_2-Antiplasmin. Circulating tPA is mostly bound to the major inhibitor PAI-1, which is a key regulator of fibrinolysis.[19] PAI-1 is synthesized in various cells including endothelial cells, megakaryocytes, and hepatocytes. In the blood and tissues, PAI-1 exists in an unstable, active form. In platelets, PAI-1 exists as a latent, more stable form.[19] Circulating PAI-1 is typically found in a complex with vitronectin, which creates a stable, active form of the molecule. Thrombin activatable fibrinolysis inhibitor (TAFI) functions as a fibrinolytic negative feedback loop by removing lysine residue sites on fibrin, preventing plasminogen binding and activation.[19] The fibrinolytic inhibitor α_2-Antiplasmin inhibits plasmin but is not very effective at inhibiting plasmin bound to fibrin. It also binds to plasminogen, impairing the interaction between plasminogen and fibrin. The liver synthesizes α_2-Antiplasmin and it has a long half-life of 3 days.[19]

Indications for Anticoagulant Use

  Anticoagulant medications are indicated for prevention of thrombosis in animals that have diseases that predispose to hypercoagulability. The diagnosis of hypercoagulability is difficult in the small animal patient and, once diagnosed, there is a paucity of information on the appropriate drugs, doses, and monitoring of these patients. Traditional coagulation testing, such as PT and aPTT, is not optimized to detect hypercoagulability and there is no evidence that decreased (e.g. shorter) values are indicative of hypercoagulability. Although a recent retrospective study suggests that shortened hypercoagulability was associated with PT or aPTT.[3] Other diagnostic tests such as AT activity and fibrinogen concentration can indicate loss of endogenous inhibitors of coagulation or increased substrate for clot formation, while measurements of concentrations of fibrin degradation products (FDPs) or D-dimers may detect excessive fibrinolysis. All of these tests, however, are insensitive indicators of hypercoagulability in dogs and cats.[23] Thromboelastography (TEG) is a test that describes the changes in whole blood during clot formation, and that allows evaluation of global hemostasis in a patient. TEG has been used to identify hypo- and hypercoagulability in dogs with immune mediated hemolytic anemia, neoplasia, and disseminated intravascular coagulation (DIC).[25-27] Diseases in small animal patients that are associated with thrombus formation and may be indications for anticoagulant therapy include pulmonary thromboembolism, portal vein thrombosis, splenic vein thrombosis, vena caval thrombosis, aortic thromboembolism, neoplasia, immune mediated hemolytic anemia, systemic inflammatory states, sepsis, DIC, protein-losing enteropathies, protein-losing nephropathies, hepatic disease, hyperadrenocorticism, and iatrogenic causes.[28] Preventing

primary feline aortic thromboembolism and recurrent thromboembolic events is one of the most common indications for anticoagulant use in general and specialty veterinary practice.

Feline Arterial Thromboembolism

The diagnosis of feline cardiac disease usually relies upon the detection of a heart murmur and subsequent echocardiographic examination. One study revealed that the prevalence of heart disease in cats with heart murmurs was 53%.[29] Alternatively, the sensitivity and specificity of the presence of a heart murmur for diagnosing cardiomyopathy was determined to be 31% and 87%, respectively.[30] In a different population of healthy cats, the prevalence of heart murmurs was found to be 41% and the prevalence of HCM was 15%.[31] The positive predictive value of a heart murmur ranged from 18-43% and was highest in older cats.[31,32] Even though the presence of a heart murmur is an established cause for further cardiac investigation, it is not considered a reliable indicator of underlying disease. Apparently healthy cats without heart murmurs constitute roughly one-third of cats with HCM.[30] Overall, it is estimated that 16% of cats in the United States will be affected with cardiomyopathy during their lifetime.[30] Feline arterial thromboembolism occurs when a thrombus forms, usually within the left atrium or left auricle, and then dislodges into the systemic circulation. Cardiac disease is the most common cause of ATE, accounting for more than 69-90% of ATE cases.[32,33] However, neoplasia and thyrotoxic cardiac disease are also associated with ATE occurrence.[32,33] Unfortunately, the clinical signs of ATE can be the first and/or only

indication of heart disease, as only 7-15% of cases have a diagnosis of pre-existing heart disease.[32,34]

The percentage of cats with hypertrophic cardiomyopathy (HCM) that are reported to develop arterial thromboembolism (ATE) ranges from 13-17%,[5,6] and 41-48% of cats have gross or histopathologic evidence of thromboembolism at necropsy.[7-9] ATE is a devastating sequela for cardiomyopathic cats since only 37% to 45% survive their first thromboembolic event.[32,33] High mortality rates have also been published for people with distal aortic/iliac thrombi.[35] However, current endovascular techniques utilizing self-expanding stents have improved the prognosis for people with aorto-occlusive disease.[36] Given the poor prognosis of ATE and the lack of an efficacious drug therapy to prevent ATE or its recurrence, euthanasia is elected in up to 35% of ATE cases.[32]

The coordination between platelets, coagulation factors, and the vascular endothelium allows thrombus formation to occur in the presence of endothelial damage, blood flow stasis, and/or hypercoagulability. The inciting factor(s) for feline arterial thrombosis formation have only been investigated in a few studies to date.[37,38] In human studies, excessive pressure or volume load and subsequent left atrial dilation leads to left atrial structural, electrical, and metabolic remodeling.[39] Left atrial structural remodeling in people includes myocyte hypertrophy, necrosis, apoptosis, and interstitial fibrosis.[39] These changes have been well-described in people with atrial fibrillation but have not been specifically studied in cats.[40] Left atrial dilation may also predispose to blood flow stasis, impaired clearance of activated circulating coagulation factors and may enhance platelet and endothelial interactions.[41] Low velocity blood flow and spontaneous echocardiographic contrast ("smoke") have been documented in cats with

cardiomyopathy and are considered risk factors for thrombus generation.[41] A hypercoagulable state has been documented with both cardiomyopathy[42] and chronic congestive heart failure[43] in people, which may predispose to thrombus formation. The overall frequency of thromboembolic events in people with reduced ejection fraction and severe congestive heart failure is 2.4% and 1.8% for women and men, respectively.[44,45] Hypercoagulability has also been suspected in dogs with chronic congestive heart failure (on the basis of elevated concentrations of fibrinogen and decreased activity of AT and protein C).[46] Both hypercoagulability and endothelial damage have been evaluated as potential mechanisms of thrombus formation in cats with cardiomyopathy.[37] In particular, hypercoagulability was described using laboratory parameters that included fibrinogen, FVIII activity, AT activity, and concentrations of thrombin-antithrombin (TAT) complexes and D-dimers (hypercoagulable cats had two or more of these criteria outside of the reference interval). Plasma concentration of vWF antigen was used as a marker of endothelial injury, with activity increasing with injury. Increased concentration of vWF antigen has been reported in people with heart disease.[47] Investigators found that 56% of cats with ATE had evidence of systemic hypercoagulability.[37] In the remaining cats with ATE, procoagulant imbalance may have been confined to the left atrium as has been demonstrated in people with mitral stenosis.[48] People with valvular heart disease, in particular, mitral stenosis, have an approximately 4% incidence of per year of systemic thromboembolism.[49] A study evaluated the levels of fibrinopeptide A and TAT within the peripheral blood, left atrium, and right atrium of people with mitral stenosis compared to normal subjects. The marker of thrombin generation, fibrinopeptide A, was increased systemically compared to normal patients. Additionally, levels were substantially higher

within the left atrium than systemically. Systemic plasma levels of TAT were not different between patients with mitral stenosis and normal subjects; however, TAT was significantly increased within the left atrium compared to peripherally.[49] This study suggests that hypercoagulablity may be confined to the left atrium in people, which may also occur in cats. Hypercoagulability (defined by decreased AT activity and increased concentrations of TAT and D-dimer) was noted, in a separate feline study, to occur in 45% of cats with HCM, and was not associated with left atrial size.[50] Increased platelet reactivity may also play a role in the genesis of thrombus formation in cats with cardiomyopathy.[51] This was assessed in a study that described an exaggerated platelet aggregation response to ADP in 10 cats with HCM, compared to 16 normal cats.

Hypoxia and inflammation can up-regulate the presence of P-selectin on the endothelial cell surface. P-selectin functions to recruit leukocytes that can express TF and promote inflammation, which may then contribute to, or initiate thrombus formation.[52] There are no studies that have evaluated the relationship between hypoxia and/or inflammation with P-selectin activity in the pathogenesis of feline ATE. Other potential components of thrombus development include Vitamin B_{12} and arginine. Plasma concentrations of vitamin B_{12} were lower in cardiomyopathic cats compared to normal cats.[38] In rat models, low plasma vitamin B_{12} concentrations result in cardiac hypertrophy and myocardial fibrosis, and in people, low plasma vitamin B_{12} concentration is considered a risk factor for coronary atherosclerosis.[53,54] Plasma levels of arginine were lower in cats with ATE than in cats with cardiomyopathy alone or healthy cats.[38] Arginine is the metabolic precursor for nitric oxide, which causes vasodilation and helps to maintain vascular tone. Nitric oxide also inhibits endothelin, platelet aggregation,

platelet adhesion, and functions to decrease the proliferation of vascular smooth muscle.[55]

Low arginine levels, and subsequent decreases in vasodilatory capacity with enhanced

platelet aggregation may contribute to the development and clinical signs related to distal

aortic thromboembolism.[38]

In approximately 71-90% of cats with ATE, the thrombotic event affects the

aortic trifurcation, resulting in bilateral occlusion of the femoral and iliac arteries.[56-58]

Embolization can occur in one or more other systemic locations such as the coronary,

brachial, mesenteric, cerebral, and/or renal arteries.[56,58] Cats that present with a single

appendicular limb affected and/or intact appendicular motor function have an increased

survival rate.[32,58] A partial occlusion of the distal aorta is also associated with an

improved survival of up to 70%.[32] Body temperature, measured per rectum, is an accurate

predictor of survival in cats with ATE, with a 50% probability of survival when the rectal

temperature is 98.9°F or above.[32] Rectal temperatures lower than 98.9°F suggest a more

compromised hemodynamic state.[58]

Variable median survival times for cats with ATE have been reported. The first

published retrospective study of 74 cats documented a median survival time of only 2

months, with no cats surviving more than 6 months post-ATE.[5] Another retrospective

study of 22 cats reported a median survival time of 11.5 months,[32] while a third

retrospective study of 43 cats showed a median survival time of approximately 6

months.[6] A larger retrospective study of 117 cats that had experienced ATE, described a

similar median survival of approximately 4 months.[33] In that study, the presence of

congestive heart failure at the time of presentation for ATE was associated with a much

shorter survival (2.5 months) compared to those without heart failure (7.5 months).[33]

More recently, a retrospective study of cats treated in general practice that survived more than 7 days post-ATE reported a median survival time of around 3 months.[59] Across all of these retrospective studies, specific therapy for the heart disease and the ATE was not standardized and was variable between practitioners. The only prospective study of cats with ATE with a standardized anticoagulant protocol compared the effect of administration of aspirin (81 mg/cat PO, q. 72 h) or clopidogrel (18.75 mg/cat PO, q. 24 h) on the time to recurrence of ATE. Cats treated with clopidogrel had a median survival time of approximately 8 months.[34]

Once ATE occurs, treatment methods consist of conservative or more aggressive therapies. It is important to note there are no studies available that clearly document the efficacy of any prophylactic therapy in preventing ATE. Conservative methods include supportive care, anticoagulant drugs, antiplatelet drugs, and waiting for endogenous thrombolytic pathways and collateral circulation to develop. Supportive care includes electrocardiographic monitoring for hyperkalemia secondary to reperfusion injury, treatment for congestive heart failure if present, and less commonly utilized medications to enhance vasodilation such as acepromazine or hydralazine. Warm compresses are sometimes used in the area of ischemia to increase vasodilation but care must be taken to not divert blood flow away from vital organs.[32,58] To help with systemic perfusion in cats without congestive heart failure, intravenous fluids can be used judiciously, or water may be given enterally through a nasogastric tube. The serotonin antagonist, cyproheptidine, may be used in an attempt to mitigate the vasoconstriction caused by serotonin released from activated platelets at the thromboembolus.[60] Serotonin and other vasoactive substances have been shown to impair collateral circulation, initiate vasoconstriction of

collateral vessels, and be integral to the clinical signs associated with ATE.[58,61,62] The feline aorta has been ligated surgically, under experimental conditions, in order to mimic a distal ATE. In these cases, collateral circulation (mostly through the vertebral blood supply) maintains blood flow to the hind limbs.[63] This and similar studies highlight the importance of the release of serotonin, prostaglandins, and other vasoactive substances in the genesis of the clinical syndrome caused by feline ATE.[58,61,62]

Vitamin K Antagonists

Anticoagulants act to slow or inhibit different aspects of the coagulation pathway. They ultimately result in the inhibition of fibrin formation. Importantly, they do not cause lysis or speed breakdown of existing clots. While anticoagulant medications may prevent the extension of a pre-existing clot, clots are typically broken down by the body's fibrinolytic system, or through medical or surgical management using local or systemic administration of thrombolytic drugs. The most common anticoagulants used for thromboprophylaxis in people and small animals are the heparins and the vitamin K antagonists (coumarin derivatives).[11,16]

Warfarin (Coumarin, Coumadin, Panwarfin) has been used in humans for prevention of venous and arterial thrombosis for many years, and is the most commonly used oral anticoagulant in people.[16,21] Warfarin administered orally to people is well-absorbed, highly protein bound, and has a long circulating half-life (37 hours) making it an attractive drug for long-term management of humans with hypercoagulability, myocardial infarction, venous thromboembolism, atrial fibrillation, and prosthetic heart

valves.[21] There is little information in small animal patients on the optimal dose of warfarin or in which specific diseases warfarin therapy is indicated.

Warfarin inhibits the activity of vitamin K epoxide reductase within the liver. Factors II, VII, IX and X are produced in the liver as inactive zymogens that must be modified by carboxylation in the presence of vitamin K so that they are able to bind calcium and participate in coagulation.[64] During this process, vitamin K is converted to vitamin K epoxide. Vitamin K epoxide reductase is required to reduce vitamin K epoxide back to the active form necessary for coagulation factor production. In the presence of warfarin, vitamin K cannot be recycled, causing a rapid depletion of vitamin K and the dependent coagulation factors. Factor VII has the shortest half-life of 6.2 hours, making the first clinical sign of warfarin activity a prolongation of the patient's PT.[64] Vitamin K is also required to produce proteins C and S. Because proteins C and S function as anticoagulants, warfarin may have both an anticoagulant and pro-coagulant effect. In people there is a rapid reduction in protein C and S activity resulting in a transient pro-coagulant state, following initiation of warfarin therapy.[23] Parenteral heparins (unfractionated or low molecular weight) are recommended in people when initiating warfarin therapy to prevent thrombus formation during this transient hypercoagulable state.[65] The most common adverse event associated with warfarin administration is hemorrhage, which may be severe. Bleeding can occur in the gastrointestinal and/or urinary tract but can also occur intra-cranially or in the pulmonary parenchyma. All of these conditions may rapidly become life threatening.

Warfarin dosing in dogs is usually initiated at 0.22 mg/kg by mouth (PO) every 12 hours.[66] In cats, the recommended starting dosage ranges from 0.06-0.09 mg/kg PO

every 24 hours.[67,68] According to one retrospective study, therapy with warfarin in cats

that survived an ATE episode was superior to aspirin for preventing recurrent ATE.[69]

However, warfarin therapy was associated with a recurrence rate and mortality rate of

43% and 32%, respectively. Moreover, warfarin use was also associated with significant

bleeding complications (manifested as epistaxis, melena, and hematuria) in up to 20% of

treated cats. Cats that receive warfarin therapy should be kept as indoor pets only to

decrease the risk of bleeding associated with injury and excessive motion. In another

study of cats with ATE due to various causes (primarily cardiomyopathies), those treated

with warfarin had median survival times that were similar to or shorter than the median

survival times of cats that were given no anticoagulant medications.[32] In addition, the

incidence of adverse bleeding events in these cats were high and included fatal

hemorrhage. In a retrospective study, warfarin treatment in 15 cats with ATE resulted in

hemorrhage in 17% of cases and 33% of these cases were euthanized within 2 weeks of

the original episode.[70] Dogs with ATE secondary to hypercoagulability or

hypofibrinolysis were administered oral warfarin adjusted to provide an international

normalized ratio (INR) of 2.0-3.0.[2] In these dogs, warfarin therapy led to improved

ambulatory function and was considered an effective treatment protocol.

 Plasma warfarin concentrations may fluctuate significantly as a result of plasma

protein levels, concurrent drug administration, and diet, leading to unpredictable

pharmacokinetics when these variables are not controlled. Due to both intraindividual and

interindividual factors, warfarin use requires frequent (initially weekly) monitoring by

either PT measurement or the calculation of the international normalized ratio (INR).

The PT test is recommended for monitoring since it is sensitive to decreased levels of FII,

FVII, and FX.[71] The INR is derived from a calculation that includes the PT of the patient,

the laboratory reference mean PT, and the relative strength of the thromboplastin reagent

(represented by the international sensitivity index [ISI] due to variability between

different reagents from different companies).[2]

$$INR = [PT_{patient}/PT_{reference}]^{ISI}$$

The use of the INR corrects for intra and inter-laboratory variability, as well as

thromboplastin reagents of varying strength, which allows for comparison across

laboratories. INR values between 2.0-4.0 are considered therapeutic in humans, and

dosing is altered based on the results.[11] The use of INR has not been validated for

veterinary patients, although its use has been reported for maintenance of dogs receiving

warfarin therapy.[2] The pharmacodynamic effect of warfarin has been evaluated in 10

healthy cats.[68] The maximum response to warfarin, as measured by PT levels, occurred

24-48 hours after a single IV dose of 0.5 mg/kg and there was a narrow therapeutic range

for efficacy of prothrombin inhibition. This study also found wide inter-individual

pharmacokinetic and pharmacodynamic responses and suggested a more tailored

approach to warfarin, similar to that used it people. The intensive monitoring required in

humans and small animal patients on warfarin may limit the number of animals in whom

this drug may be safely used.

Heparins

      Heparin is the anticoagulant chosen most frequently in veterinary medicine for

prevention of thrombus growth and thromboprophylaxis.[72,73] The heparins can be given

intravenously (IV) or subcutaneously (SC). In people, heparins can also be administered

by nebulization, causing aPTT prolongation and increased anti-FXa activity.[74] However, heparins delivered via inhalation have not been shown to produce local or systemic anticoagulant effects in dogs. Heparin has been evaluated after oral administration in dogs and was found to cause aPTT prolongation and increased anti-FXa activity after a single dose.[75] The clinical significance of orally administered heparin in dogs remains to be evaluated. Heparins have the advantage of being readily reversed, if necessary, with protamine sulfate, which binds heparins in a 1:1 ratio. There are many veterinary studies in small and large animals that assess the pharmacodynamics of heparins, but direct outcome-based information on the optimal dose of heparins for specific diseases is more limited.

Unfractionated heparin (UFH) is a heterogeneous mixture of glycosaminoglycans with molecular weights ranging from 5000-30,000 Daltons.[23] About one-third of UFH molecules contain a pentasaccharide sequence that is necessary to bind AT. The fractions of higher molecular weight heparin form a larger ternary complex that inactivates FIIa and FXa, with a ratio of 1:1 for anti-Xa to anti-IIa activity. The smaller molecular weight heparin molecules are not long enough to bind FIIa but still have affinity for FXa and have a 4:1 ratio of anti-Xa to anti-IIa activity.[11,23] Once bound to AT, UFH inhibits FIXa, FXa, FXIa, FXIIa and FIIa, with its most profound effects on FIIa and FXa. Heparin is considered an indirect anticoagulant since it works by enhancing the pre-existing properties of AT. Therefore, it works to prevent further thrombus formation. Heparin also has a direct anticoagulant effect by causing release of TFPI from the endothelial cell surface. The importance of this mechanism in disease states is not known at this time.[76]

UFH is highly protein bound and also binds to the surface of endothelial cells, macrophages and platelets.[23] The binding of UFH to endothelial cells and macrophages causes alterations in bioavailability. The effect of UFH will also be lower in conditions where AT activity is decreased. In humans, low AT activity is associated with congenital AT deficiency, acquired AT deficiency, pregnancy, severe burns, hepatic disease, nephrotic syndrome, sepsis, estrogen use, and certain chemotherapy agents.[11] Low AT levels have been reported in dogs with nephrotic syndrome and septic peritonitis.[77,78] The effect of AT activity on UFH bioavailability or pharmacodynamics has not been studied in veterinary medicine but may contribute to the observed unpredictable anticoagulant effect in both feline and canine patients.

The effects of various doses of UFH on coagulation have been studied in healthy dogs and cats.[16] Heparin is usually administered and dose adjusted by monitoring aPTT, with the goal of obtaining a value 1.5 times the normal or baseline value.[71,79,80] This goal has been extrapolated from human medicine[81] and has not been extensively studied in veterinary patients. A recent study suggests a target of 1.2-1.5 times the baseline aPTT for therapeutic effects in dogs.[82] The relationship of the aPTT value to the *in vivo* anti-Xa activity of heparins is not straightforward and is likely affected by route of administration, inherent patient variation, and the methodology of the clinical laboratory reporting the test results.[72,83] The amount of active circulating heparin is most accurately monitored by measuring anti-Xa activity levels in plasma.[80] Human recommendations for dosing of UFH are based on anti-Xa activity with an applied LMWH-specific calibration curve, targeting a therapeutic anti-Xa activity range between 0.35-0.7 U/mL.[81] Anti-Xa activity is inversely related to thrombus generation and propagation. The anti-Xa activity

that provides clinical efficacy and thrombus prevention is not known in feline or canine patients.

Given the unpredictable bioavailability and anticoagulant effect of UFH, empirical dosing recommendations for thromboprophylaxis in dogs vary from 50 to 500 U/kg given SC every 6-12 hours.[84,85] In healthy dogs given UFH at 250-500 U/kg, target anti-Xa activity of 0.35-0.7 U/mLwas achieved at all studied doses.[23] However, when UFH was given to dogs at 500U/kg SC every 8 hours, hematoma formation occurred in some dogs.[86] Dogs with immune-mediated hemolytic anemia (IMHA) may require higher doses of UFH (>300 U/kg SC) and/or more frequent dosing (every 6 hours) in order to obtain target anti-Xa levels, due to the inflammation-associated hypercoagulability of this disease.[23] In one study of 18 dogs with IMHA, administration of UFH at 300 U/kg SC every 6 hours resulted in presumed therapeutic anti-Xa levels (≥0.35 U/mL) in 44% of dogs after the first 40 hours of therapy.[79] Higher doses of UFH may also be necessary in animals with acute inflammation, due to the tendency of UFH to bind to other acute phase proteins.[79] High dose UFH (900 U/kg/d CRI) in dogs considered to be at risk for venous thrombosis resulted in hemorrhage in 4 of 6 dogs, while a lower dose (300 U/kg/d CRI) did not result in target anti-Xa activity levels.[87]

The study of UFH usage in cats has been limited. Cats with cardiac disease are at risk for ATE, but there are no studies evaluating the effects of any UFH protocol for cats with this disease. Empirical dosing of UFH ranges from 50-300 U/kg SC every 6-8 hours.[33,60] In healthy cats, an UFH dose of 250 U/kg SC every 6 hours for 5 days resulted in anti-Xa values in the therapeutic range (0.35-0.7 U/mL) in most cats for the majority of the study.[88] In a retrospective study of feline ATE, UFH administered between 50-232

U/kg SC every 6 hours, was associated with improved survival.[70] However, the cats in that study received both streptokinase and heparin therapy. This is consistent with experimental rabbit studies that showed the combination of streptokinase and UFH enhances thrombolysis in comparison to streptokinase monotherapy.[89] In a separate retrospective study, all feline ATE cases (n=100) were treated with UFH at 100-200 U/kg IV initially, then 50-100 U/kg SC every 6-8 hours.[32] The aPTT levels were maintained at 1.5-2.0 times baseline values. The regular use of UFH is likely due to extrapolation from human studies that show a benefit to early heparinization for people with peripheral thromboembolic events.[35] UFH was given to 71% of cats in another retrospective study of ATE at doses ranging from 10-300 U/kg every 6-12 hours,[33] but the effect of UFH therapy on prognosis was not assessed.

Low molecular weight heparins (LMWH) contain only glycosaminoglycans with average molecular weights near 5000 Daltons but with a heterogeneous distribution within this smaller size range. Between 25-30% of the molecules in LMWH preparations contain the pentasaccharide necessary to bind both AT and FIIa.[18,21] Due to their smaller size, LMWH exert their primary effect on FXa and their use in human patients is associated with fewer major bleeding events than UFH.[11] In people, LMWHs are effective for the prevention and treatment of venous and arterial thromboembolism.[90-93] Unlike UFH, the LMWHs are not highly protein bound and do not bind readily to endothelial cells. This makes the bioavailability of LMWH more predictable (approaching 100% in people following SC injection).[23] Due to a longer elimination half-life (3-6 hours), LMWHs only require administration every 12-24 hours in humans which make them more convenient than the UFHs.[11,23] However, the pharmacokinetic

information for an individual LMWH is not interchangeable with other LMWH preparations.[71] Compared to UFH, LMWHs bind less to macrophages which decreases the hepatic clearance of the drug. LMWHs also bind less to platelets which is associated with a decrease in the human phenomenon of heparin-induced thrombocytopenia.[94] Care must be taken in people with impaired renal function since LMWHs are mainly excreted by the kidneys. Protamine sulfate can only reverse some of the anticoagulant action of the LMWHs because anti-Xa activity is not completely neutralized.[16] The most frequently used LMWHs in veterinary medicine include dalteparin (Fragmin®, Pfizer Inc) and enoxaparin (Lovenox®, Sanofi-Aventis Inc).

Guidelines for dosing LMWHs in people for therapeutic use or for prophylactic use are tied to anti-Xa activity levels. There are several targeted dose ranges based upon different studies. The proposed anti-Xa activity range for prophylactic use (i.e., for patients without existing thrombosis who are undergoing a procedure that may predispose them to thrombus formation) is between 0.1-0.3 U/mL.[27] For people with gross evidence of thrombosis or evidence of ischemic skin lesions (due to embolic shower), the therapeutic LMWH range targets 0.5-1.0 U/mL.[81] Moreover, dosing frequency alters the targeted anti-Xa activity levels. Once-daily dosing requires a higher target anti-Xa activity level of 1-2 U/mL whereas the anti-Xa activity should be between 0.6-1 U/mL for twice-daily dosing.[95] Anti-Xa activity is generally measured at 4-6 hours after heparin administration. The efficacy of these drugs and the ideal anti-Xa activity levels for thromboprophylaxis or therapy in specific disease conditions have not been extensively studied in veterinary medicine, although evidence exists that higher overall doses of UFH might be necessary for anticoagulation in patients with diseases that result in a

hypercoagulable state.[23] One study in cats suggests that anti-Xa activity may be a poor predictor of the antithrombotic effect of LMWHs.[96]

LMWHs have been studied in dogs and cats. The expense of these drugs is still a limiting factor when using them in the clinical setting. The optimal dose and dosing interval for LMWH is not yet known in veterinary species. In dogs with TF-induced disseminated intravascular coagulation (DIC), dalteparin was given as an IV continuous rate infusion in order to produce anti-Xa activity between 0.6-0.9 U/mL. Achieving the target anti-Xa activity in this model was associated with less severe hematologic changes.[97] Eighteen dogs in an intensive care unit that were at risk for venous thrombosis received dalteparin at 100 U/kg SC every 12 hours and failed to achieve plasma anti-Xa activity greater than 0.5 U/mL.[87] Enoxaparin given to dogs at a dose of 0.8 mg/kg SC every 6 hours showed consistent anti-Xa activity within the range of 0.5-2.0 U/mL for the length of the study period (36 hours).[98]

The bioavailability of dalteparin in cats after SC injection is approximately 100%, with rapid absorption and elimination, resulting in the need for frequent injections.[99] Dalteparin given to cats at a dosage of 100 U/kg SC every 12 hours did not reliably achieve target anti-Xa activity (0.3-0.6 U/mL).[100] Based upon results in this study of healthy cats, dalteparin may require administration every 6 hours in order to maintain anti-Xa activity within the target range.[99] However, anti-Xa activity may not reflect clinical thromboprophylactic efficacy. Human and veterinary studies have shown beneficial results of LMWHs even when the anti-Xa activity is below the target range or undetectable.[96,101] This may be due to the effects of LMHWs on platelet function, release of TFPI, and enhanced fibrinolysis.[99] Results from another study in healthy cats

confirmed that LMWHs need to be dosed frequently to maintain anti-Xa activity within the presumed therapeutic range. In this study, dalteparin given at a dosage of 150 U/kg SC every 4 hours and enoxaparin given at a dosage of 1.5 mg/kg SC every 6 hours provided more reliable target anti-Xa activity in this population.[88] A retrospective study evaluated the use of dalteparin in 43 cats with underlying cardiomyopathy.[90] This study was not designed to assess the efficacy of dalteparin in reducing thrombotic events or improving survival, but did provide evidence that home SC administration was feasible. Bleeding events were extremely rare, necessitating the discontinuation of dalteparin in only one cat. Overall, dalteparin is well tolerated in cats but has not been shown to prevent occurrence, reduce severity, or decrease frequency of ATE in cats.[90]

Similar to dalteparin, enoxaparin has rapid absorption and elimination. In a prospective pharmacokinetic study in healthy cats, all subjects obtained target anti-Xa activity after the second dose of 0.75 or 1.0 mg/kg SC every 6 hours.[102] In cats that received multiple doses (for 4 days), there was no appreciable accumulation of anticoagulant activity after the second dose. Clearance of enoxaparin in this study was found to be slightly slower than that of dalteparin. A dosage schedule for enoxaparin of 0.75 mg/kg every 6 hours was recommended to maintain anti-Xa activity within the target range for thromboprophylaxis. Enoxaparin was used in a venous stasis model in healthy cats and was found to decrease thrombus development when given less frequently (1 mg/kg SC every 12 hours).[96] In a prospective study of healthy cats, suboptimal anti-Xa activity (below target concentrations) was found after administration of enoxaparin at 1.0 mg/kg SC every 12 hours and dalteparin administered at a dosage of 100 U/kg SC every 12 hours.[88] In contrast, cats in this study treated with UFH at a dosage of 250 U/kg SC

every 6 hours had more consistent anti-Xa activity within or above the therapeutic range.

Another FXa inhibitor chemically similar to the heparins, requiring parenteral

administration, is fondaparinux (Arixtra®, GlaxoSmithKline). Fondaparinux was found

to be superior to enoxaparin for prevention of venous thromboembolism in people after

knee surgery or hip-fracture surgery.[103,104] Fondaparinux was safely administered to 6

healthy cats at two different dosages (0.06 and 0.20 mg/kg SC every 12 hours).[105] The

most beneficial dosage for LMWHs to prevent thromboembolic disease is not known and

will require prospective, outcome-based studies.

Direct Thrombin Inhibitors

Direct thrombin inhibitors are small molecules that bind to thrombin (Factor IIa)

at one of two sites,[106] and are indicated in human patients for prevention of arterial or

venous thrombosis, primarily in patients who have experienced heparin-induced

thrombocytopenia (HIT) or other heparin-related complications. This class of drugs

includes argatroban (licensed under no trade name, GlaxoSmithKline), dabigatran

(Pradaxa®, Boehringer Ingelheim), lepirudin (Refludan®,Bayer), bivalirudin

(Angiomax®, The Medicines Company), and ximelagatran (Exanta®, AstraZeneca). The

direct thrombin inhibitors have activity against fibrin-bound thrombin and also

circulating thrombin. They also do not require AT as a co-factor for this inhibition.[106]

They are attractive alternatives for thromboprophylaxis because they do not have as many

drug interactions as warfarin and they do not require intensive monitoring.[107]

Ximelagatran has been withdrawn from the market due to significant hepatotoxicity.[108]

Dabigatran has been shown to be non-inferior to warfarin for prevention of

thromboembolism and stroke in people with atrial fibrillation, but the cost of this drug has remained prohibitive.[109] Argatroban is available for IV administration, and has been studied in an experimental dog model of cardiopulmonary bypass. This study found that argatroban was safe and decreased coagulation system activation while dogs were on cardiopulmonary bypass circuits.[110] There are no published clinical studies of direct thrombin inhibitor usage in veterinary species.

Factor Xa Inhibitors

Oral FXa inhibitors (e.g., rivaroxaban and apixaban) are novel anticoagulants that have been approved for use in people for the prevention of venous thromboembolism after total hip replacement, total knee replacement, for the prevention of stroke with atrial fibrillation, and for the prevention of recurrent ischemic events after acute coronary syndrome.[13,4,111-114] These agents were developed to overcome the disadvantages of the vitamin K antagonists and heparins. As previously noted, disadvantages of warfarin include a wide variability in dose response, a slow onset of therapeutic effect, a narrow therapeutic window, multiple food and drug interactions, and the need for frequent monitoring.[107] Heparins require parenteral administration that can make compliance challenging for both short and long-term use. Whereas the vitamin K antagonists reduce levels of FII, FVII, FIX, and FX, the FXa inhibitors are selective antagonists of the active site of FXa, considered a key portion of the coagulation cascade. FX is located at the convergence point of the intrinsic and extrinsic coagulation pathways. Activation of FX is the rate-limiting step for thrombin generation, with one molecule of FXa producing up to 1000 thrombin molecules.[115] The FXa inhibitors have activity against both free and

prothrombinase-bound FXa (FXa complexed with FVa).[116] The prothrombinase complex surpasses free FXa in its ability to activate prothrombin to thrombin and increases the reaction rate for thrombin formation by approximately 300,000 fold.[117] The inhibition of these complexes is ideal and leads to decreased thrombin generation and decreased thrombin induced activation of platelets. Moreover, the FXa inhibitors are 10,000 times more selective for binding factor Xa than other proteases and they do not require binding to AT to exert this effect.[14,118,119]

The antithrombotic efficacy of RVX was evaluated in an experimental rabbit model of venous thrombosis.[120] Multiple oral doses of RVX were administered and a dose-dependent decrease in thrombus formation was observed. This study also evaluated IV RVX administration found it to be as efficacious as PO administration with both routes resulting in decreased thrombus growth. The safety, pharmacodynamics, and pharmacokinetics of a range of single, oral RVX doses were evaluated in healthy male subjects.[14] In this phase I dose-escalation study, subjects were administered 1.25 mg, 5 mg, 10 mg, 20 mg, 40 mg, and 80 mg doses in a crossover design. No major adverse events, including bleeding events, occurred at any of the doses tested, and the incidence of side effects was similar between RVX and placebo. The pharmacodynamics of RVX were evaluated with FXa activity, PT, aPTT, HepTest (a LMWH activity assay), AT activity and thrombin activity.[14] There was a dose-dependent inhibition of FXa activity and prolongation of PT, aPTT, and HepTest. Maximum prolongation of FXa activity was noted between 1-4 hours after PO administration. RVX did not have a significant effect on either AT or thrombin activity. The pharmacokinetics of RVX were evaluated by HPLC/MS and were also found to be dose-dependent, showing a strong correlation

between the pharmacodynamic and pharmacokinetic findings. In particular, there was a strong, direct relationship between RVX plasma concentrations, inhibition of FXa activity, and PT prolongation. More specifically, PT prolongations of 1.2-2.1 times baseline values, aPTT prolongations of 1.2-1.7 times baseline values, and inhibition of FXa activity ranging from 20% to 61% were observed with RVX doses ranging from 5-to-80 mg.[14] Corresponding plasma RVX concentration ranged from approximately 60 µg/L-290 µg/L. The maximum PT and aPTT elevations resulted from peak plasma RVX concentrations of 316 µg/L (range 185-532 µg/L). Two other phase I dose-escalation studies confirmed the predictable, dose-proportional pharmacokinetics and pharmacodynamics of RVX with no accumulation beyond that achieved at steady state.[121,122] PT prolongation was correlated linearly with RVX plasma concentrations and had lower inter-individual variability compared to aPTT and FXa-activity levels. These findings reveal that PT levels may be the most reliable test to determine RVX exposure.[121]

The bioavailability after oral RVX administration in people is approximately 80%.[14] Bioavailability in rats and dogs after oral administration is reported to be 57-66% and 60-86%, respectively.[115] It is quickly absorbed in many species, typically reaching peak plasma levels within 30 minutes (dogs and rats)[115] to 1-4 hours (humans).[14,122,123] The half-life of RVX is short in rats (IV $t_{1/2}$ = 0.9 hours, PO $t_{1/2}$ = 1-2 hours) and dogs (IV $t_{1/2}$ = 1 hour, IV $t_{1/2}$ = 0.9 hours).[115] In people, it has a longer half-life of 5-9 hours after PO administration.[122,123] RVX is highly, reversibly bound to plasma proteins, mostly to albumin and to a lesser extent to α_1-glycoprotein. The amount of unbound RVX differs among species with an unbound fraction of 1.3% in rats, 6.5% in mice, 23.4% in rabbits,

10.4% in dogs, 7.1% in pigs, 18.3% in monkeys, and 5.1% in people.[115] Studies of tissue

distribution show RVX does not accumulate in the body after a wide range of single and

multiple daily doses.[115] The pharmaceutically active molecule of RVX remains

unchanged in the plasma, with no active major circulating metabolites. In rats, dogs, and

people, RVX is eliminated primarily unchanged through both renal and fecal-biliary

routes.[116] The degree of renal versus fecal excretion differs among species. In dogs, the

amount of urinary excretion is less (52%) than that found in people (66%) while fecal

excretion is more in dogs (43%) than in people (28%). Because the primary route of

excretion in people is renal, RVX requires dose adjustment in the presence of decreased

creatinine clearance rates. A smaller amount of RVX metabolism is through oxidative

degradation and hydrolysis.[116] A study utilizing high-performance liquid chromatography

(HPLC) with mass spectrometry (MS) identified M-1 as the main excreted metabolite in

people.[116] The M-1 metabolite is eliminated via both the fecal and urinary routes. Other

metabolites, M-4 and M-7 were also found in smaller, almost equal quantities. The

metabolite M-4 is eliminated only via the urinary route whereas M-7 is found in both

urinary and fecal excreta.

Bleeding complications have been reported with all anticoagulants. For FXa

inhibitors, there are no currently available reversal agents. For mild bleeding,

discontinuation of the drug may be adequate due to the short half-life of these drugs.

Supportive care with activated charcoal, gastric lavage, and mechanical compression can

be instituted for treatment of hemorrhage.[124] Andexanet alfa (Portola Pharmaceuticals) is

a specific FXa inhibitor antidote that has been developed but is not yet commercially

available.[125] Andexanet alfa is a recombinant protein analog of FXa that exhibits dose-

dependent reversal of FXa inhibition.[125,126] A phase 4 study is currently underway to evaluate andexanet alfa in patients that present with major bleeding events.

The RVX once daily oral direct factor Xa inhibition compared with vitamin K antagonism for prevention of stroke and embolism trial in atrial fibrillation (ROCKET AF) study was designed to compare the efficacy of RVX versus dose-adjusted warfarin for the prevention of stroke in high-risk patients with non-valvular atrial fibrillation.[127] In people, the risk of ischemic stroke is increased by 4-5 times in the presence of atrial fibrillation. Vitamin K antagonists are recommended to decrease the rate of systemic thromboembolism in people with atrial fibrillation. In the ROCKET AF study, RVX was found to be non-inferior to warfarin for prevention of ischemic stroke and systemic embolism (P<0.001). Furthermore, there was a decreased risk of thromboembolism in patients treated with RVX (P=0.12). Bleeding events were similar between groups, but the risk of fatal bleeding and intracranial hemorrhage was decreased in patients receiving RVX. Patients receiving warfarin were less likely to have major bleeding from gastrointestinal sites. A noted study limitation was that patients on warfarin had documented INR values within the therapeutic range (2-3) a mean of 55% of the time. However, in subgroup analysis, there was no difference in outcome when centers with the best INR control were compared to those with the poorest.[127] Arterial thrombosis and embolization has been associated with atrial fibrillation in three dogs.[128]

The efficacy of RVX for secondary thrombus prevention in patients with acute coronary syndrome was evaluated in the anti-Xa therapy to lower cardiovascular events in addition to aspirin with or without thienopyridine therapy in subjects with acute coronary syndrome – thrombolysis in myocardial infarction trial (ATLAS ACS-TIMI).[13]

Approximately 10% of patients suffering from acute coronary syndrome will suffer cardiovascular death or a repeat ischemic event in the year following their first episode. This recurrent event rate occurs in the setting of antiplatelet therapy with aspirin monotherapy or with aspirin combined with a thienopyridine (e.g., clopidogrel). The ATLAS ACS-TIMI study had four treatments arms: aspirin plus RVX, aspirin plus placebo, aspirin and a thienopyridine plus RVX, and aspirin and a thienopyridine plus placebo. RVX was administered for 6 months after the initial incident in once daily or twice daily doses of 5mg, 10mg, and 20mg. Clinically significant bleeding that required medical intervention increased in a dose-dependent fashion with RVX. This bleeding was more common in patients also receiving aspirin and a thienopyridine. RVX administration was associated with a trend towards a reduction in recurrent ischemic events. Additionally, it was found to decrease the risk of death, myocardial infarction, or stroke in patients receiving aspirin monotherapy (P=0.0270). There was no additional benefit of RVX for patients receiving dual antiplatelet therapy.

The efficacy of RVX for prevention of deep vein thrombosis and pulmonary embolism after orthopedic surgery was evaluated in the regulation of coagulation in orthopedic surgery to prevent DVT and PE trials (RECORD 1-4).[129-132] The RECORD 1 trial compared RVX (10 mg daily) versus enoxaparin (40 mg daily) for 5-9 days after total hip replacement.[129] RVX reduced the primary endpoint of DVT, non-fatal PE, or death by 78% compared to enoxaparin and was found to be non-inferior and superior to enoxaparin (P<0.001). In the RECORD 2 trial, a more extended treatment regimen of RVX (10 mg PO daily for 36 days) was compared to enoxaparin (40 mg SC daily for 14 days) in humans patients undergoing total hip replacement surgery.[130] Prolonged RVX

therapy was associated with a risk reduction of 7.3% for DVT, nonfatal PE, and all-cause mortality (P<0.0001). Major clinical bleeding events were similar between enoxaparin and RVX-treated patients. The RECORD 3 trial evaluated the efficacy of therapy with RVX (10 mg PO daily) versus enoxaparin (40 mg SC daily) for 10-14 days after total knee replacement surgery.[131] The primary endpoints of proximal and/or distal DVT, nonfatal PE, and death occurred in 9.6% of patients treated with RVX and 18.9% of patients receiving enoxaparin (P<0.001). This study established both the non-inferiority and superiority of RVX for thromboprophylaxis after knee replacement. Lastly, the RECORD 4 trial investigated the efficacy of RVX (10 mg PO daily) versus enoxaparin (30 mg SC twice daily) for 5-9 days in human patients after total knee replacement surgery.[132] The primary endpoint of DVT, nonfatal PE or death occurred in 6.9% and 10.1% of patients taking RVX or enoxaparin, respectively (P=0.0118).

Acute venous thromboembolism (DVT or PE) occurs commonly in people with an annual incidence rate of 1-2 cases per 1000.[133] The cause may be idiopathic or thrombosis may occur secondary to neoplasia, surgery, trauma, or pregnancy.[134] Recurrent thrombotic events are common, necessitating anticoagulant use for a minimum of 2 weeks with some patients requiring life-long therapy.[135] The incidence of venous thromboembolic recurrence is approximately 11% within 1 year of anticoagulant discontinuation, 29% within 5 years, and 40% within 10 years.[136] Several studies were designed to evaluate the efficacy of RVX in preventing embolic disease in these patients. The oral RVX for symptomatic venous thromboembolism (EINSTEIN-DVT) study investigated the use of RVX for prevention of recurrent, symptomatic DVT.[133] RVX or the combination of enoxaparin and warfarin was administered for 3, 6, or 12 months.

RVX was non-inferior to standard therapy (P<0.001). The EINSTEIN-PE study assessed the use of RVX for prevention of pulmonary embolism.[137] This study design was similar to EINSTEIN-DVT in that it compared the use of RVX to enoxaparin and dose-adjusted warfarin for 3, 6, or 12 months. RVX was found to be non-inferior and associated with less major bleeding than standard therapy for PE (P=0.003). The EINSTEIN-Extension study enrolled patients that had already received treatment for venous thromboembolism for 6-12 months.[134] In addition, there was uncertainty over whether longer-term treatment with an anticoagulant would be beneficial in these patients. Patients received either RVX (20mg PO once daily) or placebo. Symptomatic recurrent VTE occurred in 1.3% of those treated with RVX and 7.1% of those receiving placebo (P<0.001). There was a moderate increase in bleeding complications compared to placebo. This study provides evidence that RVX is suitable for thromboprophylaxis in patients at risk for VTE.

Since RVX has selective effects on FXa and predictable pharmacology, frequent coagulation monitoring is not considered necessary in humans who take the drug. Pre-clinical *in vivo* and *in vitro* studies of the small-molecule, selective, direct FXa inhibitor RVX (Xarelto®, Bayer Healthcare), showed dose-dependent inhibition of FXa activity that corresponded to prolongations of PT and aPTT.[117] RVX exhibits a concentration-dependent prolongation of PT and aPTT, but the concentration dependent increase in aPTT typically requires supratherapeutic doses.[138] When compared to PT, the aPTT test is considered less sensitive for FXa inhibitors; however, the effect of RVX on PT values is still considered weak. Clinically, PT testing may be beneficial in cases with hemorrhagic complications. The ability of PT to detect low drug concentration and prolongation varies widely based upon differential sensitivity of thromboplastin reagents

used in the assay.[139] Dilute PT (dPT) may be better able to monitor FXa inhibitor drugs.

This test utilizes a diluted thromboplastin reagent that increases the sensitivity of the

assay.[138,139] The dPT test may create an *in vitro* environment that is more physiologic

than standard PT testing. Chromogenic assays are available that measure the

concentration of FXa within the sample. These results are compared to a standard assay,

typically with a known amount of inhibitor, and from this comparison, the amount of FXa

inhibitor can be determined.[140] The concentration of RVX within a sample can be

measured with the use of specific RVX calibrators and controls.

 In vitro studies of the anticoagulant effect of RVX in canine and feline blood

indicate that these drugs have similar anticoagulant effects as seen in humans.[141,142] In

pooled canine platelet-poor plasma, *in vitro* addition of RVX resulted in a concentration-

dependent prolongation of coagulation parameters.[143] PT and aPTT times were not as

sensitive as thrombin generation and anti-Xa activity for detection of the anticoagulant

effect of RVX. There are no prospective studies regarding the efficacy of RVX in

reducing the primary endpoint of thrombosis in veterinary species.

 Apixaban (Eliquis®, Pfizer/Bristol-Myers Squibb) is another oral FXa inhibitor

that appears safe with an acceptable adverse event profile in humans. An *in vitro* human

model showed that apixaban can also inhibit platelet aggregation through the prevention

of thrombin generation via the tissue factor coagulation pathway.[144] Studies in people

with deep vein thrombosis or that require thromboprophylaxis for potential systemic

thromboembolism after knee replacement surgery have shown apixaban to be as effective

as or superior to currently used anticoagulants.[145-147] The pharmacokinetics and

pharmacodynamics of apixaban after PO and IV administration have been evaluated in

healthy cats.[57] Cats received 0.2 mg/kg PO once and then received a single dose of 0.2

mg/kg IV after a washout period. Importantly, there were no adverse events associated

with a single dose of apixaban. Peak anti-Xa activity was reached at 4 hours post-oral

administration and a direct correlation was found between apixaban concentration and

anti-Xa activity. Bioavailability was high (85.5%) and similar to the bioavailability found

in other species.[148] The half-life of apixaban in healthy cats was 3.3 hours, which is

considerably shorter than the 12-hour half-life found in people.[149] By 6 hours post-oral

administration, anti-Xa activity returned to baseline. Further prospective studies with

apixaban in healthy cats are needed in order to discover the optimal dose and dosing

schedule for clinical efficacy.

Antiplatelet Agents

The pathogenesis of ATE involves platelet activation, adhesion, aggregation and

propagation along with activation of coagulation factors. In people, thrombotic

complications associated with atherosclerosis are common. Platelet activation may

contribute to vessel occlusion and ischemic events. Platelets have also been shown to be

an important source of inflammatory proteins such as platelet derived growth factor and

transforming growth factor-β that can cause progression of atherosclerotic disease.[150] The

mainstay of treatment for prevention of arterial thrombosis in people has been aspirin

therapy. Aspirin is an irreversible inhibitor of cyclooxygenase (COX), producing an

effect that lasts the lifetime of the platelet. Both the COX-1 and COX-2 enzymes catalyze

the conversion of arachidonic acid released from membrane phospholipids to

prostaglandins (PGH_2, PGE_2, $PGE_{2\alpha}$), prostacyclin (PGI_2), and thromboxane A_2

(TXA$_2$).[71] Platelets preferentially transform PGH$_2$ to TXA$_2$, which causes

vasoconstriction and induces platelet aggregation. Vascular endothelial cells convert

PGH$_2$ to prostacyclin (PGI$_2$) which causes vasodilation and inhibits clot formation.[71] The

COX-1 isoform is more involved with TXA$_2$ production within platelets.[71] The COX-1

and COX-2 isoforms are both involved with PGI$_2$ production in vascular endothelial

cells.[71] Aspirin has 50-100 times more affinity for platelet-derived COX-1 than COX-2

derived from monocytes or endothelial cells. Human patients receiving long-term once

daily low-dose aspirin (81 mg PO daily) have complete TXA$_2$ inhibition while

maintaining the ability of other cells to produce PGI$_2$. Much higher doses of aspirin are

required to inhibit the synthesis of PGI$_2$.[71] Aspirin was found to decrease mortality

secondary to acute myocardial infarction at a similar rate to the thrombolytic drug

streptokinase in the second international study of infarct survival trial (ISIS-2).[151] The

widespread use of aspirin ensued after studies showed patients given aspirin versus

placebo had a 25% relative risk reduction for vascular death, myocardial infarction or

stroke.[152] The half-life of aspirin varies widely between cats and people. In cats, the half-

life is around 38 hours whereas in people the half-life is considerably shorter at 15-20

minutes.[71] Based upon the survival benefit in people, aspirin was traditionally

recommended for initial treatment and secondary prevention of feline ATE. The

recommended dose was 81 mg per cat PO every 48-72 hours.[153] An *in vitro* study of

healthy cats showed that arachidonic acid-induced platelet aggregation decreased when

cats were treated with either aspirin or aspirin and diltiazem.[154] Cats receiving aspirin at

81 mg every 3 days PO were found to have gastrointestinal side effects at a rate of 22%

and an ATE recurrence rate of 28%.[33] Retrospective studies have reported the overall

median survival time for ATE recurrence with aspirin therapy as ranging from 24-149 days.[33,34,59,60] A low dose aspirin regimen consisting of 5 mg per cat every 72 hours PO was investigated due to the possibility that higher dose aspirin caused decreased endothelial cell prostacyclin production, with resultant decreased platelet antagonism and less vasodilation. No significant difference was found between cats receiving high dose or low dose aspirin for prevention of ATE recurrence.[33] However, low dose aspirin was associated with fewer gastrointestinal side effects. Further research into the effects of aspirin on feline platelets revealed that a dose of 5 mg/kg per cat every 48 hours PO did not affect platelet aggregation.[155] Thromboxane levels did decrease after this aspirin dose suggesting that robust platelet aggregation can occur in cats with relatively low concentrations of TXA_2 present. Furthermore, the release of other second messengers such as ADP and/or 5-HT may provide redundant signaling pathways for platelet aggregation.[155] Aspirin therapy may provide less of a benefit in feline ATE compared to myocardial infarction in people because the pathogenesis behind these two diseases is distinctly different. Myocardial infarction in people is commonly due to coronary artery disease caused by the build up of atherosclerotic plaques and platelets.[150] In cats with cardiogenic ATE, the disease is a result of a dislodged central thrombus or a fragment of the thrombus without underlying atherosclerotic coronary artery disease. The most direct pathophysiologic comparison of feline ATE is to thromboembolic events related to atrial fibrillation in people.[34]

The thienopyridines are a class of platelet inhibitors that prevent ADP-induced platelet activation through irreversible antagonism of the specific platelet ADP receptor, $(P2Y_{12})$.[34,156,157] Collateral blood flow around the site of ATE is reduced in the presence

of serotonin. The thienopyridines decrease serotonin levels by decreasing platelet activation. Since serotonin has been linked to the reduced collateral circulation that follows ATE, the subsequent decrease in serotonin levels from thienopyridine administration may offer an additional benefit. Examples of thienopyridines include clopidogrel and ticlopidine. Hepatic biotransformation is required of both drugs to create an active metabolite. Therefore, the plasma concentration of the parent compound does not correlate with antiplatelet activity produced by the metabolite. The use of ticlopidine in people has been associated with a reduced incidence of thromboembolic stroke.[158] Ticlopidine was evaluated in healthy cats at several different oral dosages.[159] Consistent reduction in platelet aggregation was only found at the highest dose tested (250mg PO) but this dose was associated with significant side effects such as anorexia, vomiting and weight loss. The frequency and severity of side effects prohibited the clinical usefulness of this agent for cats. The use of clopidogrel in people has fewer side effects than ticlopidine and provides enhanced efficacy.[157] Landmark studies of clopidogrel versus aspirin or dual therapy consisting of clopidogrel and aspirin in people with symptomatic atherosclerotic disease showed that therapy with clopidogrel is associated with a decreased risk of stroke, myocardial infarction, ischemia events, and/or overall vascular death.[160,161] Clopidogrel at a dose of 18.75mg PO every 24 hours decreases platelet aggregation in healthy adult cats.[156] A separate study in healthy adult cats showed that clopidogrel at all doses tested (75mg, 37.5mg, and 18.75mg PO daily) had significant antiplatelet effects as measured by whole blood aggregometry and oral mucosal bleeding time.[162] The aforementioned study also showed that clopidogrel was safe and well-tolerated in cats. Clopidogrel administration at the three listed doses did not cause any

adverse gastrointestinal signs, hemorrhagic events, or alterations in clinicopathologic

values. Hepatic injury has been reported along with clopidogrel administration in

people[163] but this was not found after short-term (7 days) therapy in cats.[162] The

secondary prevention of cardiogenic arterial thromboembolism in the cat: the double-

blind, randomized, positive-controlled feline arterial thromboembolism; clopidogrel vs.

aspirin trial (FAT CAT) study evaluated clopidogrel versus aspirin therapy for secondary

prevention of cardiogenic ATE in cats.[34] Cats received either clopidogrel (18.75 mg per

cat PO every 24 hours) or aspirin (81 mg PO every 72 hours). Significantly fewer

recurrent ATE events were seen in cats receiving clopidogrel, compared to those

receiving aspirin (49% vs. 75%, P=0.024). In addition, cats receiving aspirin had a

shorter median time to recurrence than those receiving clopidogrel (192 days vs. 443

days). No adverse effects were noted with clopidogrel treatment in this study. The FAT

CAT trial is the only prospective study to document the efficacy of a prophylactic therapy

for prevention of feline ATE. Due to this study, clopidogrel is currently recommended for

the secondary prevention of cardiogenic ATE.[34] There are no studies to document the

efficacy of antiplatelet agents for the primary prevention of cardiogenic ATE. However,

clopidogrel is often prescribed to cats considered at high risk for embolic disease.

Another class of antiplatelet drugs act as antagonists against the glycoprotein

(GP) IIb-IIIa receptor on the platelet surface. GP IIb-IIIa activation leads to platelet

aggregation regardless of the agonist used and also leads to binding of fibrinogen and von

Willebrand factor (vWF) on the platelet surface.[150] Examples of this class of GP IIb-IIIa

antagonists include eptifibatide, abciximab, and tirofiban. These drugs are primarily used

in people for percutaneous coronary interventional procedures and for acute coronary

syndrome.[150] In the European/Australian stroke prevention in reversible ischemia trial (ESPRIT) and the evaluation of abciximab for the prevention of ischemic complications trials (EPIC), GP IIb-IIIa inhibitors were shown to decrease overall mortality and ischemic events but were associated with a larger risk of bleeding complications.[164,165] Feline platelet function was impaired by eptifibatide in an *in vitro* model of healthy cats.[166] However, the *in vivo* assessment of eptifibatide as a constant rate infusion in cats with experimentally induced arterial injury caused unpredictable circulatory failure and sudden death.[167] The significant adverse events found in this study preclude the clinical use of eptifibatide in the cat.

Thrombolytic Agents

The use of local or systemic plasminogen activators to target thrombus dissolution and return circulatory flow is common and effective in people with thromboembolic events. These agents have been investigated in feline ATE with suboptimal results. A specific strain of beta-hemolytic streptococci produces streptokinase (Kabikinase, Streptase). Streptokinase results in fibrinolysis by forming stable complexes with plasminogen that promote plasmin formation.[71] The activation of plasmin by streptokinase does not require fibrin as a co-factor. Streptokinase has the potential to cause excessive bleeding due to its non-fibrin specific activity that includes the degradation of prothrombin, FV, FVII, and FXII.[168] The streptokinase-plasminogen complex facilitates activation of both fibrin-bound plasminogen and circulating plasminogen. Patients can have pre-existing antibodies from previous streptococcal infections that require loading doses to overcome the inhibition and obtain a full

therapeutic effect.[71] Three separate studies have described the effects of streptokinase

administration to cats. In an experimental model of ATE, streptokinase administration

caused complete thrombus dissolution in two cats and a reduction in thrombus size in all

cats.[169] When streptokinase was administered to six cats with ATE and two cats with left

atrial thrombi, all eight cats died during drug administration.[170] A retrospective study of

streptokinase administration to 46 cats with ATE revealed a survival rate of only 33%.[70]

Moreover, 39% of these patients died during hospitalization and 28% were euthanized

due to complications associated with streptokinase administration or due to poor response

to treatment. There was no difference between survivors and non-survivors and the time

of streptokinase administration after the onset of clinical signs. Overall, streptokinase

administration does not improve prognosis and can cause significant, life-threatening side

effects. Urokinase, similar to streptokinase does not require fibrin as a co-factor for the

activation of plasminogen. It is an endogenous thrombolytic agent, produced by renal

cells and found as a natural component of urine.[71] Minimal information regarding the

efficacy of urokinase in cats is available. An experimental model of ATE in healthy cats

revealed that escalating doses of a urokinase infusion, from 4,000-250,000 U/hour,

resulted in increased PT and aPTT values.[171] Two case reports document the use of

urokinase in cats with cardiac disease and secondary ATE. One cat was diagnosed with

mitral stenosis and bacterial myocarditis and died during treatment with urokinase,

furosemide, antibiotics, and IV fluids.[172] In a separate case report, a cat with HCM and

secondary ATE was treated with systemic IV urokinase and no improvement was

noted.[173] Due to poor response, local intra-arterial delivery of urokinase was administered

via a catheter delivered through the carotid artery. Angiographic evaluation of the

thrombus showed resolution and motor function returned 2 days post-local urokinase administration. No side effects such as hemorrhage or hyperkalemia were noted in this single case.

Tissue plasminogen activator has more specific activity towards clot-bound cross-linked fibrin than circulating fibrinogen. Less breakdown of circulating fibrinogen occurs with tPA than with streptokinase or urokinase due to the specific tPA binding site on thrombus bound fibrin.[168] The use of tPA in people has been shown to restore arterial patency and improve outcome if administered within 3-6 hours of an acute myocardial infarction or acute ischemic stroke.[174,175] A prospective study evaluating tPA in 11 cats with ATE was terminated early due to adverse outcomes.[176] In that study, all cats developed adverse effects that included azotemia, hyperkalemia, acidosis, neurologic signs, cardiac arrhythmias and/or sudden death. The administration of tPA was associated with effective thrombolysis as measured by improved pulse quality and/or motor skills. However, the survival rate in this study was 27%, which prevents the recommendation of tPA for feline ATE.

CHAPTER 3

IN VITRO EFFECTS OF RIVAROXABAN ON FELINE COAGULATION INDICES

Introduction

It is estimated that 16% of cats in the United States will be affected with

cardiomyopathy in their lifetime.[30] The percentage of cats with hypertrophic

cardiomyopathy (HCM) that are reported to develop arterial thromboembolism (ATE)

ranges from 13-17%,[5,6] and 41-48% of cats have gross or histopathologic evidence of

thromboembolism at necropsy.[7-9] Current available treatment protocols aimed at

secondary thromboprophylaxis in cats include the use of antiplatelet agents such as

aspirin or clopidogrel, and anticoagulant medications such as unfractionated (UFH) or

low-molecular weight heparins (LMWH). There are no studies to document the efficacy

of any drug therapy for primary prevention of systemic thromboembolism in cats. The

use of aspirin, UFH, or LMWH has not been shown to prevent recurrent ATE or improve

survival after an embolic event.[33,60,90] In addition, the inconvenience of parenterally

administered UFH or LMWH may limit their usage and may substantially decrease client

compliance. The frequency of administration of both UFH and LMWH is another

deterrent to their clinical long-term usage. The administration of clopidogrel (a

thienopyridine antiplatelet drug) after cardiogenic ATE is associated with a reduced rate

of recurrent embolism and a longer median time to recurrence.[34] However, the recurrence

rate remains high with 36% of cats having a repeat embolic event in the first year despite

clopidogrel therapy.[34] Given the high ATE-associated morbidity and mortality in cats with cardiac disease, there is a need for a safe, effective, predictable, easily administered thromboprophylactic anticoagulant for chronic use.

Rivaroxaban (RVX) is a new oral, direct inhibitor of Factor Xa that holds promise for use as chronic anticoagulant therapy. To date there are published studies in rat, rabbit, and dog models.[14,115,117,120] Human trials, such as the ROCKET-AF trial, demonstrated that RVX was non-inferior to warfarin for the prevention of stroke or systemic thromboembolism caused by non-valvular atrial fibrillation.[127] In addition, the EINSTEIN-DVT and EINSTEIN-PE trials compared RVX to warfarin and LMWH for the treatment of deep vein thrombosis and pulmonary embolism and found RVX to be non-inferior.[133,137] In rats, dogs, and humans, RVX is cleared primarily unchanged through renal and fecal-biliary routes with smaller amounts metabolized through hydrolysis and cytochrome p450 pathways.[115] There have been no studies of RVX in cats, although slower hepatic metabolism and glucuronidation pathways may prolong the plasma half life and duration of effect to the benefit of convenient dosing in this species.[122]

RVX has the potential to limit growth of in situ ATE, and potentially to decrease overall clot formation in cats, making it an attractive drug for management of feline ATE. In humans, both prothrombin time (PT) and partial thromboplastin time (aPTT) become prolonged with increasing plasma drug concentrations of RVX.[112,113] Even though PT times prolong in a concentration dependent manner with higher plasma RVX concentrations, therapeutic levels of RVX have minimal effects on PT and results can be variable.[139] Additionally, aPTT times can be prolonged with RVX administration but

most consistently they are prolonged at supratherapeutic RVX concentrations.[139]

Thromboelastography (TEG) can provide information on global hemostasis and can be

used to identify both hypocoagulability and hypercoagulability.[1] TEG tracings consist of

a reaction time (R time) that corresponds to the time elapsed, in minutes, from the start of

the test to the onset of clot formation, and evaluates the intrinsic pathway in an

unactivated sample.[177] A prolonged R time indicates slower initiation of clot formation.

The K time is an assessment of the rapidity of clot formation and is measured in minutes

from the end of the R time to the point where the tracings reach a distance spanning

20mm.[88] Prolonged K times can be indicative of hypocoagulable states, and can be

altered due to deficiencies in FII, FVIII, platelet count, platelet function, fibrinogen

concentration, hematocrit, and thrombin formation.[177] The α-angle is a representation

(angle) of the speed at which the clot forms, with higher values corresponding to more

rapidly forming clots. The maximum amplitude (MA) value is a depiction of the final

strength of the clot.[88] Weaker clots should display decreased MA values. The use of

LMWH prolongs TEG tracings in cats[88], and may be an adjunctive diagnostic tool to

assess anticoagulant efficacy or overdose with anticoagulant medications. A study

evaluating TEG for RVX anticoagulation monitoring in people showed statistically

significant alterations in R time, K time, and α angle at high concentrations of RVX.[178]

RVX did not cause MA values to change from baseline control values.[178] The effects of

RVX on TEG parameters in cats have not been studied to date.

The effects of RVX on coagulation parameters have not been studied in cats.

However, *in vitro* studies are feasible since the stable drug is the parent compound and

requires no further *in vivo* metabolism. The primary goal of this study was to evaluate the

in vitro effect of multiple concentrations of RVX in feline citrated whole blood on standard coagulation testing (PT, aPTT, anti-factor Xa activity [anti-Xa]) and TEG. Our hypothesis was that RVX would show a concentration dependent prolongation of PT, aPTT, dilute prothrombin time (dPT) and an increase in anti-Xa activity with a concentration-dependent progressive hypocoagulability on TEG. The secondary goal was to provide data that could inform *in vivo* testing and ultimately lead to clinical trials in cats predisposed to ATE.

Methods and Materials

Blood from five purpose-bred, adult, male domestic shorthair cats was collected in accordance with AAALAC guidelines. This study was approved by the University of Georgia Animal Care and Use Committee. The cats were deemed healthy based upon results of a physical exam, complete blood count (CBC), serum biochemistry analysis, urinalysis, and normal coagulation status. These cats were part of an existing research colony and were free from all drugs for a period of at least 60 days prior to the start of this study. All cats were sedated with 2 mg/kg ketamine[b] and 0.5 mg/kg of midazolam[c] intravenously immediately prior to blood collection. Using a 19-gauge butterfly catheter[d], whole blood was collected from each cat into 1.8 mL tubes containing 3.2% sodium citrate[e] for a final citrate:blood ratio of 1:9.

---

[b] Ketamine 10mg/mL, Hospira, Lake Forest, IL
[c] Versed 5mg/mL, Hospira, Lake Forest, IL
[d] Exel International, Los Angeles, CA
[e] BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ

Pure RVX compound was isolated from commercial Xarelto® tablets[f] and verified by high-pressure liquid chromatography tandem mass spectrometric (LC-MS/MS) analysis at the Auburn University Specialized Pharmaceutical and Experimental Center for Translational Research. RVX was dissolved in dimethyl disulfoxide in order to create RVX fortifying solutions. These RVX fortifying solutions were added to aliquots of citrated blood obtained from five healthy adult cats to achieve final plasma RVX concentrations of 0, 40, 100, 160, 220, 500, 1000, 2000, 4000, 6000, and 8000 µg/L.[113,129] Not all coagulation parameters were tested at all RVX concentrations. Plasma RVX concentrations were based on human pharmacokinetic data for doses that demonstrated efficacy in preventing venous thromboembolism in people undergoing total hip or knee replacements. Human studies have found that total daily doses in the range of 5 mg to 20 mg PO have a good safety profile and good efficacy in preventing venous thromboembolism in people undergoing total hip and total knee replacements.[112,123,131] A separate study has correlated these doses to plasma concentrations ranging from 60 to 290 µg/L depending on dose and frequency of treatment.[14,117,122,123] These concentrations provided initial target concentrations for testing in this study.

Twenty minutes after blood collection, RVX at 0 µg/L, 40 µg/L, 160 µg/L, 220 µg/L, 500 µg/L, 1000 µg/L, 2000 µg/L, 4000 µg/L, 6000 µg/L, and 8000 µg/L was added to the citrated tubes. The tubes were gently inverted five times and then rested for 10 more minutes. After 30 minutes, 500 µL of whole blood from each of the different RVX concentration tubes was added to a kaolin vial and gently inverted three times. An aliquot

---

[f] Xarelto 10mg tablets, Bayer HealthCare AG, Leverkussen, Germany

of this mixture (340 µL) was placed in a TEG cup along with 20 µL of calcium chloride. TEG analysis was then performed.

The remainder of the blood was centrifuged at 2700 rpm and 4°C for 10 minutes. Citrated plasma was removed and frozen at -20°C until batched analysis was performed at the Comparative Coagulation Section of the Animal Health Diagnostic Center at Cornell University (coagulation analysis). *In vitro* plasma RVX concentrations ranging from 0 to 8000 µg/L were assessed using plasma coagulation testing (PT, dPT, and aPTT). In addition, a modification of the Rotachrom assay (Diagnostica Stago), using dalteparin as an index compound, was used to assess the FXa inhibitory action (anti-Xa) of the drug. The coagulation assays were selected from those shown to be sensitive in pharmacodynamic studies of RVX in human subjects.[138]

Statistical Methods

Descriptive statistics were generated using a commercially available statistical software program.[g] Data were tested for normality using the Kolmogorov-Smirnov test. Sequential values were compared using a one-way ANOVA for repeated measures. The difference in the dPT, PT, aPTT, or anti-Xa values among the different plasma RVX concentrations was evaluated by pairwise multiple comparison procedures (Holm-Sidak method) because the data was not normally distributed. P values <0.05 were considered significant. Normally distributed data are reported as the mean +/- one standard deviation. Non-parametric data are reported as median (minimum value – maximum value).

---

[g] Prism v.6, GraphPad Software Inc., La Jolla, CA

Results

Samples from 5 healthy male cats demonstrated a concentration-dependent prolongation of dPT, PT, and aPTT and a concentration-dependent increase in anti-Xa activity (Table 3.1). Selected results of the kaolin-activated TEG of feline whole blood, at differing RVX concentrations, are listed in Table 3.2. Hypocoagulability would be expected to prolong the R time, prolong the K time, and decrease both the angle and the MA value. The addition of RVX to whole blood had a statistically significant effect on the TEG parameters R time (P<0.001), K time (P<0.001), and α angle (P<0.001). However, there was no statistically significant effect of RVX on the MA value (P=0.38). A dose-dependent increase in the R time was noted, but only at concentrations of 220 µg/L and greater (Figure 3.1). Statistically significant prolongations of TEG R time from baseline did not occur until concentrations of 2000 µg/L (P=0.001) were reached. Significant increases were also noted between 160 µg/L and both the 6000 µg/L (P=0.001) and 8000 µg/L (P=0.001) and between the 220 µg/L and the 8000 µg/L (P=0.001). The K time (Figure 3.2) was prolonged with increasing doses of RVX over 220 µg/L. However, statistically significant change was only noted from baseline at the highest RVX concentration tested, 8000 µg/L (P=0.001). The α angle (Figure 3.3) decreased with increasing doses of RVX over 220 µg/L. Significant decreases (all P values=0.001) occurred between baseline and 2000 µg/L, 6000 µg/L, and 8000 µg/L. Significant decreases in the angle values between 160 µg/L and 8000 µg/L (P=0.001) and between 220 µg/L and 8000 µg/L (P=0.001) were also present. The MA decreased (Figure 3.4) with increasing doses of RVX but only at RVX concentrations above 6000

µg/L. There was no statistical significance in MA values among the RVX doses (P=0.383). Overall, *in vitro* RVX caused dose-dependent hypocoagulability changes in TEG parameters at RVX concentrations of 220 µg/L and greater; however, kaolin-activated TEG did not appear to be sensitive to identify low concentrations of RVX in the cat.

Table 3.1: Mean +/- standard deviations for *in vitro* plasma coagulation parameters at different concentrations of rivaroxaban. The asterisks denote statistically significant changes from the baseline plasma RVX concentration of 0 µg/L.

Coagulation assays (mean and SD) at different rivaroxaban concentrations in cats				
Assay (reference plasma)	Rivaroxaban Concentration (µg/L)			
	0	**160**	**220**	**2000**
aXa (U/ml)	0	1.0 ±0.3*	1.7 ±0.5*	4.1 ±0.2*
dPT (18.3 s)	18.5 ±0.8	24.9 ±1.4	28.4 ±2.7	80.1 ±6.6*
PT (12.1 s)	11.4 ±0.6	not available	23.8 ±7.0*	65.7 ±8.5*
aPTT (18.2 s)	15.6 ±2.4	17.0 ±3.9	21.9 ±4.7	39.6 ±4.4*

Table 3.2: Mean TEG parameters (standard deviation) at various rivaroxaban plasma concentrations in feline blood. The asterisks denote statistically significant changes from the baseline plasma RVX concentration of 0 µg/L.

Thromboelastography measures of feline whole blood at differing rivaroxaban concentrations				
	Plasma rivaroxaban Concentration (µg/L)			
	0	**160**	**220**	**2000**
R time (min ±SD)	3.5 ±0.7	4.4 ±1.2	5.4 ±3.0	16.0 ±9.1*
MA (mm ±SD)	61.2 ±4.7	60.3 ±2.9	64.8 ±5.3	61.3 ±4.2
K (mm ±SD)	1.1±0.1	1.5±0.26	1.55±0.78	3±1.6*
α angle **(mm ±SD)**	73.9±0.75	68.4±2.9	69.2±9.3	53.6±12.1
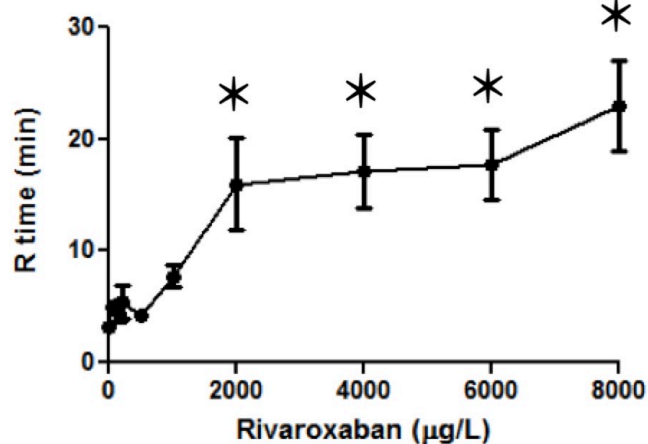
Figure 3.1: Thromboelastography (TEG) mean R (reaction) time parameter with error bars as a function of rivaroxaban concentration in feline whole blood *in vitro*. The R time is the time elapsed in minutes from the beginning of the test to the onset of blood clotting. The asterisks denote statistically significant changes from baseline.
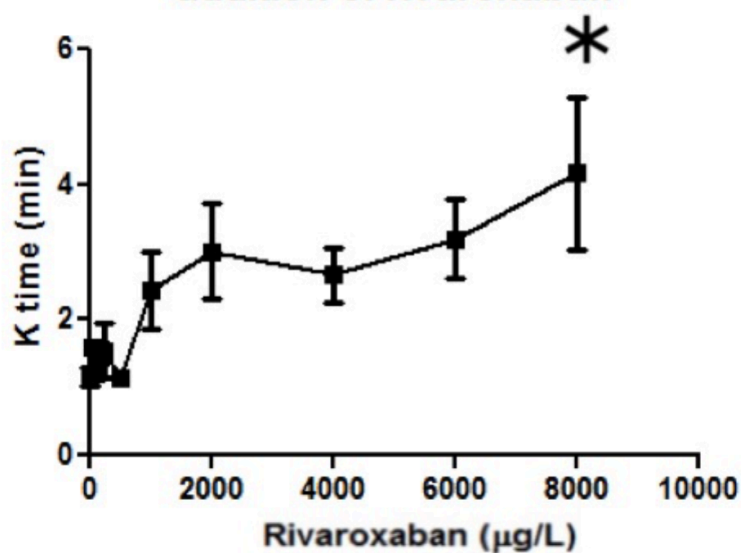
Figure 3.2: Thromboelastography (TEG) mean K time with error bars as a function of rivaroxaban concentration in feline whole blood *in vitro*. The K parameter is a measurement of clot strength and is measured from the split on the TEG tracing to the time it takes for the clot to produce 20-mm amplitude of resistance. The asterisk denotes the only statistically significant change from baseline.

TEG Angle in citrated feline blood with in vitro addition of rivaroxaban
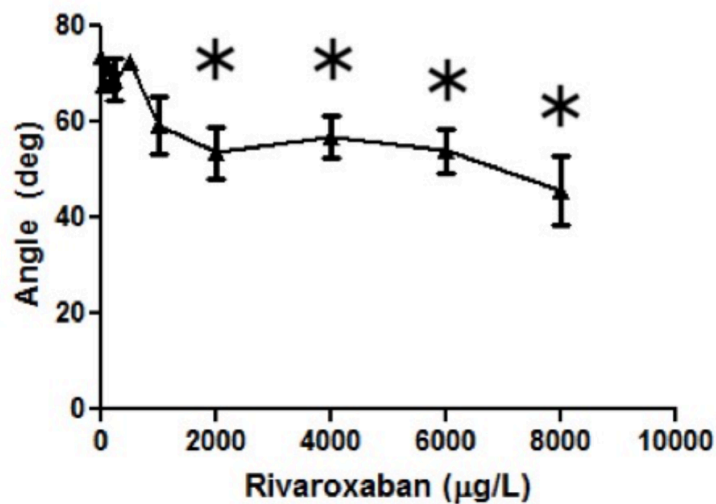
Figure 3.3: Thromboelastography (TEG) mean angle with error bars as a function of rivaroxaban concentration in feline whole blood *in vitro*. The angle is an additional indicator of clot strength and correlates to the rate of thrombin activity catalyzing the conversion of fibrinogen into active fibrin. The asterisks denote statistically significant changes from baseline.

**TEG MA in citrated feline blood with in vitro addition of rivaroxaban**
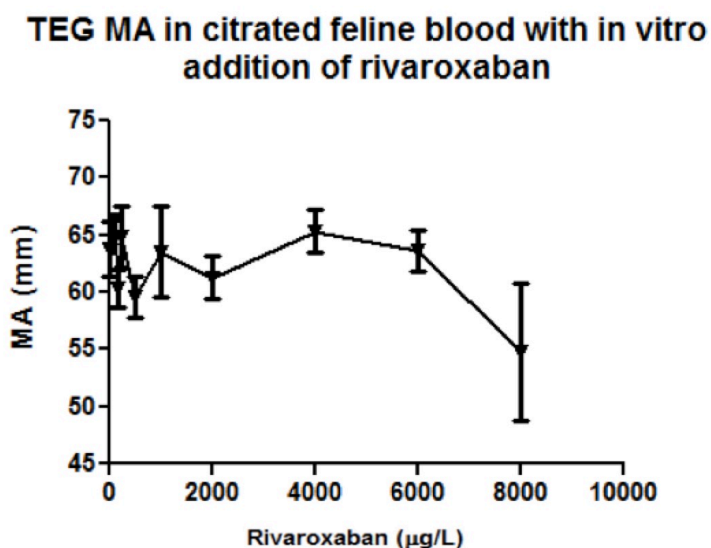
Figure 3.4: Thromboelastography (TEG) mean MA (maximum amplitude) with error bars as a function of rivaroxaban concentration in feline whole blood *in vitro*. The MA occurs once the clot is entirely formed and is the maximum distance between the diverging lines on the TEG tracing which reflects clot strength. No statistically significant changes from baseline were noted.

Evaluation of plasma coagulation parameters (dPT, PT, aPTT, and anti-Xa) revealed prolongations at all RVX concentrations, but these prolongations were most pronounced at concentrations above 220 µg/L (Figures 3.5, 3.6, 3.7, 3.8 and Table 3.1).

Anti-Xa levels between 0.5-1.0 U/mL were achieved at *in vitro* plasma RVX

concentrations between 160 and 220 µg/L (Figure 3.8). At 220 µg/L, the dPT times were

not prolonged compared to baseline (29.2 +/- 4 sec vs. 18.5 +/- 0.8 sec, P = 0.148), nor

were aPTT values prolonged compared to baseline (21.9 +/- 5 sec vs. 15.6 +/- 2 sec, P =

0.07). Significant prolongations of dPT from baseline were observed at the 500 µg/L

concentration, however (60.4 +/- 42 sec, *P* = 0.005).
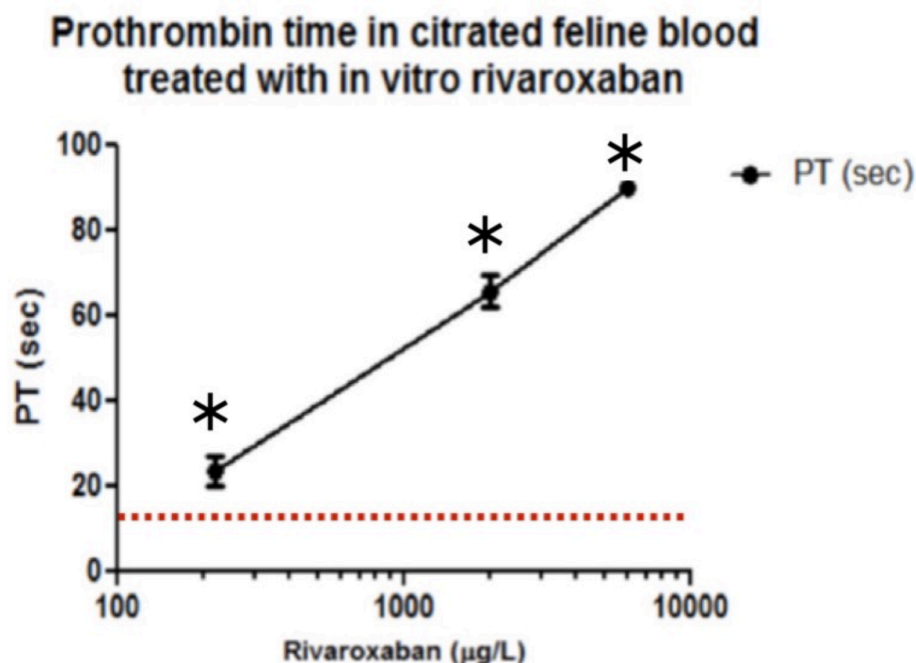
Figure 3.5: Mean prothrombin time with error bars as a function of rivaroxaban

concentration (plotted on a logarithmic scale) in feline whole blood *in vitro*. The dashed

line represents the control PT of 12.1 seconds. The asterisks denote statistically

significant changes from baseline.
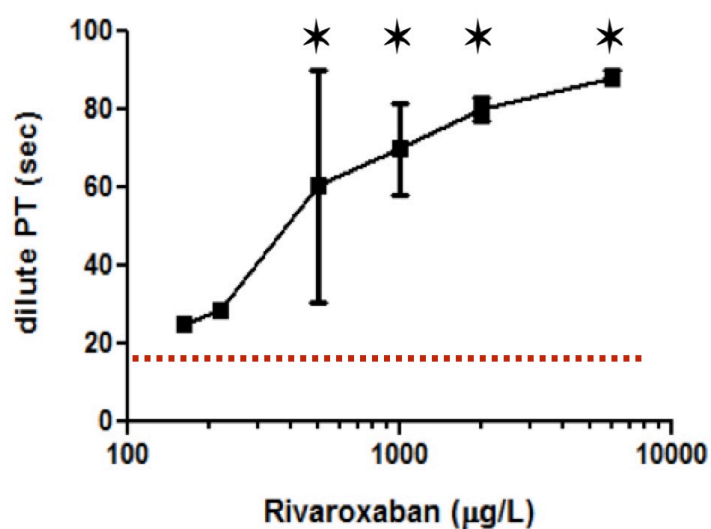
Figure 3.6: Mean dilute prothrombin time with error bars as a function of rivaroxaban concentration (plotted on a logarithmic scale) in feline whole blood *in vitro*. The dashed line represents the control dPT of 18.3 seconds. The asterisks denote statistically significant changes from baseline.
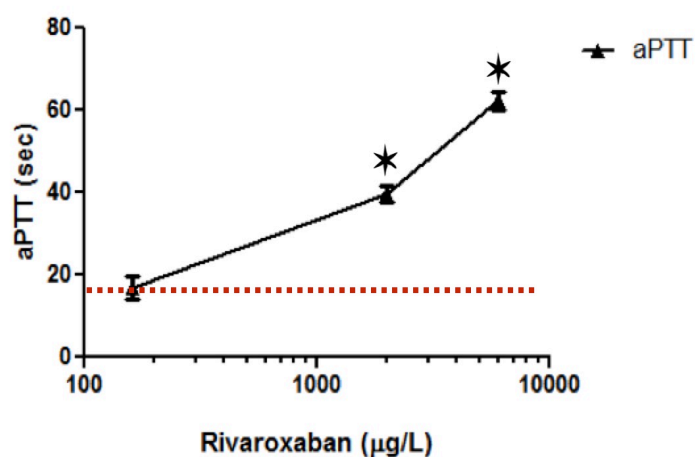
Figure 3.7: Mean activated partial thromboplastin time with error bars as a function of rivaroxaban concentration (plotted on a logarithmic scale) in feline whole blood *in vitro*. The dashed line represents the control aPTT of 18.2 seconds. The asterisks denote statistically significant changes from baseline.
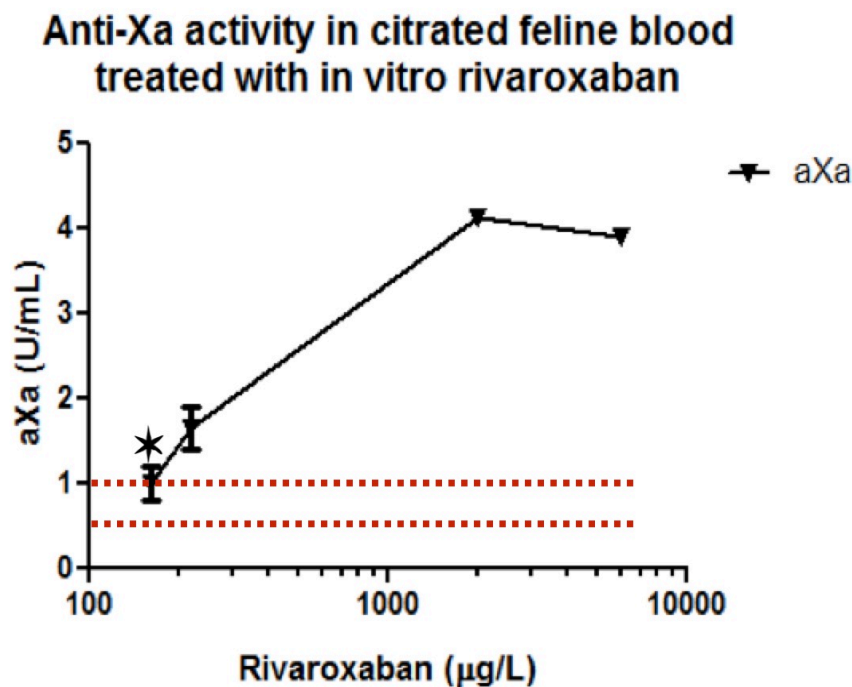
Figure 3.8: Mean anti-Xa activity with error bars as a function of rivaroxaban

concentration (plotted on a logarithmic scale) in feline whole blood *in vitro*. The dashed

line represents the target anti-Xa of 0.5-1 U/mL. The asterisk denotes the *in vitro* plasma

RVX concentration that achieved the targeted anti-Xa activity.

Discussion

The results of this study suggest that healthy cats show a dose response to RVX

that is similar to other species. Also, it appears from this data that dPT, PT and aPTT

prolongations in cats with whole blood concentrations of RVX between 60-290 μg/L

correlate to similar prolongations of these measures seen in people given therapeutic

doses of RVX, making these tests of questionable utility for identifying these low drug

concentrations. However, TEG parameters did change significantly from baseline at high RVX plasma concentrations, which may indicate it could be used to identify potential overdosage in cats.

The ideal anticoagulant to be used for cats at risk for ATE would not interact with other drugs or foods, and would have predictable pharmacokinetics and pharmacodynamics, be administered orally, and be associated with minimal adverse events. In this *in vitro* study, RVX was shown to have predictable anticoagulant activity in healthy cat plasma and exerts similar dose dependent effects on feline coagulation assays *in vitro* as it does in people. In particular there was a sustained, dose-dependent prolongation of PT and dPT and dose-dependent increase in anti-FXa activity. The dPT assay may be more sensitive at detecting lower RVX concentrations than the PT assay, as it is in people but this was not specifically studied. Further studies would be required to determine if the dPT test is more sensitive to RVX concentration effects that the PT test. The aPTT test was only mildly prolonged from baseline at differing plasma RVX concentrations and was not sensitive for detection of low concentrations of RVX.

TEG can provide information on global hemostasis.[1] Since initial studies showed a wide variation in feline TEG normal values, kaolin was used as an activator for coagulation in this study.[179] R values were expected to increase with increasing levels of RVX and values did increase in a dose-dependent fashion (>220 μg /L). However, R values were not reliably different from baseline until high plasma RVX concentrations (>2000 μg/L) occurred. The K time was prolonged and the angle decreased with increasing RVX concentration, although significant changes were not seen until high RVX concentrations. The MA value did not appear to be helpful for multiple RVX

concentrations and did not change significantly before plasma RVX levels reached 8000 µg/L. Plasma RVX concentrations ranging from 60-290 µg/L correlate well with effective anti-Xa activity in people.[111-113,123] Based upon these results, kaolin-activated TEG does not appear to be sensitive to relevant concentrations of RVX in the cat.

The initial target plasma concentrations were chosen for this study based upon correlate human target levels that provide effective thromboprophylaxis. The PT, dPT, and aPTT prolongations in healthy cats with plasma concentrations of RVX between 160 to 220 µg/L correlate to similar prolongations of these measurements seen in people given therapeutic doses of RVX for prevention of both arterial and venous thromboembolism. Therefore, RVX may be a beneficial drug in the future for cats with cardiogenic thromboembolism but further *in vivo* studies are necessary.

Limitations of the study include the examination of RVX in only a healthy cat population. However, dose-dependent effects need to be elucidated in a healthy cat population in order to extrapolate to a high-risk population. No conclusions can be drawn regarding the use of this particular FXa inhibitor in cats that are considered at risk for ATE. We can only conclude that RVX exerts a linear dose-dependent inhibition of coagulation in healthy cats *in vitro*. Echocardiograms were not performed on the cats in this study prior to blood collection in order to assess for occult cardiac disease. Therefore, it is possible that some cats had underlying disease. Cardiac disease, if present, could have altered coagulation parameters obtained at different plasma RVX concentrations. TEG was not a sensitive diagnostic tool for assessing RVX effects at presumed therapeutic plasma concentrations. Variability in TEG results has been noted between operators.[1,180] Only one operator performed the TEG in this study, which decreases the

possibility of interindividual variability. Other causes for variable TEG results include traumatic venipuncture, increased storage time, *in vitro* hemolysis and red cell mass.[1] Care was taken to prevent traumatic venipuncture and samples were not grossly hemolyzed. All samples had TEG initiated following a 30-minute rest period, and all cats had normal red cell mass assessed by a complete blood count.

In conclusion, RVX has similar *in vitro* coagulation effects in healthy cats as it does in other species and may play a role in feline thromboprophylaxis. Monitoring of anticoagulant effects can be done through measurement of dPT, PT, anti-Xa activity, and plasma drug concentration. However, dPT and anti-Xa activity, in addition to direct plasma drug concentration measurement, are the most sensitive detectors of low plasma RVX concentrations. Kaolin-activated TEG does not appear to be sensitive to low concentrations of RVX in the cat. Further *in vivo* studies are warranted based upon this initial data.

CHAPTER 4

PHARMACODYNAMIC EFFECTS OF INTRAVENOUS RIVAROXABAN IN TWO

CATS

Introduction

Rivaroxaban (RVX) is an orally administered, direct inhibitor of FXa that does

not require interaction with antithrombin for anti-Xa activity, which distinguishes it from

the heparins.[14] The anticoagulant effects of RVX in people at risk for thromboembolic

disease are as robust as standard therapy with warfarin and/or LMWH when used for

prevention of arterial or venous thromboembolism.[13,24,129,131,133,134,137] Due to its

predictable pharmacokinetics in other species, the efficacy for preventing systemic

thromboembolism, and the minimal side effects associated with administration, RVX has

great potential for long-term use in cats with high-risk for arterial thrombus formation. In

addition, there are currently no oral anticoagulants for use in the cat that are safe, with a

predictable effect or with straightforward monitoring protocols.

An *in vitro* pilot study determined the effects of plasma concentrations of RVX

ranging from 0 to 8000 μg/L using standard plasma coagulation testing (PT, dilute PT

[dPT], and aPTT), chromogenic anti-Xa activity, and thromboelastography (TEG).

Human pharmacokinetic data targeted a peak plasma RVX concentration of 222.6 μg/L

for prevention of venous thromboembolism in people undergoing total hip or knee

replacements.[112,131] The plasma coagulation assays showed dose-dependent increases at all RVX concentrations. Compared to baseline, there were statistically increased PT and dPT times and anti-Xa activity at RVX concentrations above 220 µg/L. These results suggest that cats have a similar dose response to RVX as other species, and based on anti-Xa and coagulation data, the drug effectively achieves the targeted changes in anti-Xa levels and coagulation times at plasma concentrations between 40 and 220 µg/L. Based upon these preliminary positive results, this study was designed to determine the pharmacodynamics and safety of RVX in two healthy cats after single IV doses. Our hypothesis was that IV administration of RVX would result in prolongation of dPT, PT, and aPTT levels and be well tolerated in cats.

Methods and Materials

Two purpose-bred adult, male, domestic shorthair cats were housed in accordance with AAALAC guidelines. The cats were deemed healthy based upon results of physical examination, complete blood count, serum biochemistry analysis, coagulation profile, and urinalysis. Cats were evaluated a minimum of eight-times daily for adverse events including bleeding, anorexia, and other gastrointestinal signs. In addition, cats were evaluated 30 hours post-RVX administration to assess for any longer term side effects.

The day prior to drug administration, cats were sedated using a combination of dexmedetomidine[h] (5 µg/kg IV) and butorphanol[i] (0.2 mg/kg IV), and jugular venous

---

[h] Dexdomitor, Zoetis, Florham Park, NJ
[i] Torbutrol 10mg/mL, Zoetis, Florham Park, NJ

catheters[j] were placed percutaneously using a modified Seldinger technique. Catheters were flushed with 50% dextrose[k] when not in use for sampling, and flushed with 0.9% saline[l] during sampling times. A waste sample of 3 mL was withdrawn from the catheter (priming volume of 0.7mL), before study samples were collected.

Commercially available 10 mg RVX tablets[m] were crushed and dissolved in DMSO to obtain a concentration of 0.6 µg/µL. Based on our *in vitro* preliminary data, target anti-Xa activity was presumed to be obtained at plasma drug levels between 100 and 220 µg/L. A bolus of 64 µg/kg was administered IV over 5 minutes via a cephalic catheter,[n] to keep it separate from the catheter used for sampling of blood for coagulation analysis. Blood samples for coagulation analysis (2.7 mL) were collected into 3.2% sodium citrate (final ratio 1:9 citrate:blood) at baseline, 15, 30, and 60 minutes, and at 3, 6, 24 and 30 hours after RVX administration.

Results

There were no adverse reactions associated with IV RVX administration. No bleeding complications occurred throughout the study period. Both PT and aPTT times were prolonged after IV RVX administration in one cat, however, the second cat in the study only showed prolongations of PT times. The PT time was prolonged from baseline starting 15 minutes after administration, which also corresponded to the peak PT prolongation in both cats. The PT time was not significantly different from baseline by 6

---

[j] 20 ga. 13 cm single lumen catheter, Arrow Teleflex International, Raleigh, NC
[k] Dextrose 0.5g/mL, Hospira, Lake Forest, IL
[l] 0.9% Sodium Chloride 1000mL, Hospira Inc, Lake Forest, IL
[m] Xarelto 10mg tablets, Bayer HealthCare AG, Leverkussen, Germany
[n] Exel International, Los Angeles, CA

hours post-administration in one cat, while the other cat had mild PT prolongations 24 hours after RVX administration. The dPT demonstrated similar prolongations to PT with peak prolongation occurring at 15 minutes. The anticoagulant effect of RVX as demonstrated by dPT results above the control dPT, were appreciable until the 6 hour sample (Figure 4.1). The aPTT values were more variable, with one cat having no prolongations noted at any time point, and the other cat showing peak prolongation at the 15-minute time sample. In the cat that exhibited prolonged aPTT values, the aPTT returned to baseline by 6 hours post-administration.
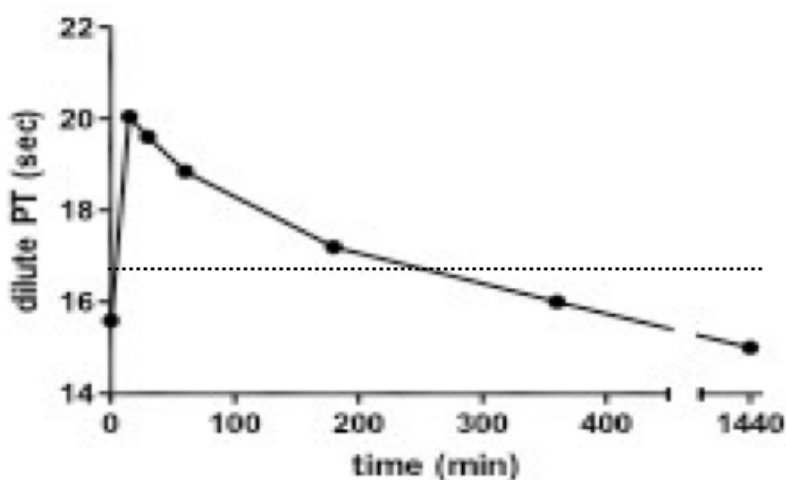
Figure 4.1: Dilute prothrombin time in 2 cats treated with 64 μg/kg IV rivaroxaban. The dashed line represents the control dPT value of 16.7 seconds. dPT time returned to normal by 6 hours post-IV administration.

Discussion

This pilot *in vivo* study of 2 cats demonstrated that IV RVX was safe and effective in prolonging the coagulation times in cats. A previous *in vivo* study of a GP IIb/IIIa platelet antagonist showed unpredictable circulatory failure and sudden death after IV administration after positive *in vitro* results.[167] Importantly, no adverse events were noted in the two cats tested in this study. No minor or major bleeding complications occurred after administration. Further IV testing would be required in a larger population of healthy cats before concluding that RVX has an acceptable safety profile, although the drug is designed for oral, not IV usage, so this is probably more relevant. Moreover, testing in cats with pre-existing renal and hepatic disease would be necessary to determine the safety in those sub-populations. Cats have lower cytochrome P-450 activities and lower glucuronidation capacity which may result in a longer half-life of RVX than in other species and in healthy cats.[181] Further dosing studies are required to determine the side effects and appropriate dosing interval for cats for effective thromboprophylaxis.

IV dosing of RVX caused prolongation of coagulation times, as expected. The dPT and PT tests appear to be more sensitive to the anticoagulant effects of RVX than aPTT. This is consistent with our previous *in vitro* study that evaluated RVX on coagulation parameters in feline plasma. Further investigation into the *in vivo* effects of oral and IV RVX are warranted.

CHAPTER 5

PHARMACOKINETIC AND PHARMACODYNAMIC EVALUATION OF ORAL

RIVAROXABAN IN HEALTHY ADULT CATS[1]

Abstract

Objectives: To determine the pharmacodynamics and pharmacokinetics of rivaroxaban in healthy cats and to evaluate the clinicopathologic effects of various plasma rivaroxaban concentrations within the target therapeutic ranges established for human beings.

Design: Prospective randomized cross-over study performed between July 2013 and November 2014.

Setting: Veterinary university teaching hospital

Interventions: Cats were treated with oral rivaroxaban at single, fixed doses (1.25mg, 2.5 mg, 5 mg PO); q. 12 hours for 3 days (1.25mg); q. 24 hours for 7 days (2.5mg) and q. 24 hours for 28 days (1.25mg). Blood samples were collected for CBC, blood chemistry, and rivaroxaban anticoagulant action based upon prolongation of dilute prothrombin (dPT), activated partial thromboplastin time (aPTT), FXa inhibition [anti-Xa activity (aXa)] and LC-MS/MS determination of drug concentration.

Measurements and Main Results: The cats had no signs of hemorrhage or clinicopathologic off-target adverse effects. There were dose dependent prolongations of coagulation times and an increase in aXa, with peak effect at 3 hours post-administration. There was a direct correlation between plasma rivaroxaban concentration and DPT and aXa. Coagulation parameters returned to baseline by 24 hours after the last dose.

Conclusions: Oral rivaroxaban was well tolerated by healthy cats with predictable pharmacokinetics and anticoagulant effects. Clinical studies of rivaroxaban are warranted in cats with heart disease.

Abbreviations:

aPTT - activated partial thromboplastin time

aXa - anti-Xa activity

dPT - dilute prothrombin time

LMWH - low-molecular weight heparin

LC-MS/MS – high pressure liquid chromatography tandem mass spectrometry

PT - prothrombin time

RVX – rivaroxaban

UFH - unfractionated heparin

Introduction

It is estimated that 16% of cats in the United States will be affected with cardiomyopathy during their lifetime.[1] The percentage of cats with hypertrophic cardiomyopathy (HCM) that are reported to develop arterial thromboembolism (ATE) ranges from 13-17%,[2,3] and up to 41% of cats affected with HCM have gross or histopathologic evidence of thromboembolism at necropsy.[4] [4] ATE is a devastating sequela for cardiomyopathic cats since only 37% to 45% survive their first thromboembolic event.[5,6] Currently available treatments for thromboprophylaxis in cats include the use of antiplatelet agents such as aspirin and/or clopidogrel or anticoagulant medications such as unfractionated heparin (UFH) or low-molecular weight heparin (LMWH). These anticoagulant therapies have not improved survival or prevented

recurrence of thromboembolism,[7,8] although a recent abstract of a prospective,

randomized, multicenter clinical trial comparing clopidogrel and aspirin for the prevention

of thromboembolism suggested that cats that recovered from an initial ATE event and

subsequently received clopidogrel lived for a longer time before thromboembolic

recurrence or cardiac death compared to those that received aspirin.[2] The high thrombosis-

associated morbidity and mortality of cats with cardiac disease substantiates the need for a

safe, effective, predictable, easily administered thromboprophylactic anticoagulant for

chronic use.

Rivaroxaban (RVX) is an orally administered direct inhibitor of activated Factor

X (FXa)[9] that holds promise for use in chronic anticoagulant therapy. Orally administered

RVX is absorbed rapidly in humans, reaching maximum plasma concentrations 3 hours

after ingestion, with a half-life of 4-9 hours.[10] The ROCKET-AF trial, which studied

RVX and warfarin for the prevention of stroke or systemic thromboembolism in human

patients with non-valvular atrial fibrillation demonstrated that RVX was non-inferior to

warfarin for this indication.[11] In addition, the EINSTEIN-DVT trial compared RVX to

warfarin and LMWH for the treatment of deep vein thrombosis in people and found RVX

to be non-inferior.[12] RVX is currently FDA approved in the United States for

prophylaxis of deep vein thrombosis in human orthopedic surgical patients and for

prevention of stroke in patients with non-valvular atrial fibrillation. In human studies, a

peak plasma RVX concentration (C_{max}) of 140-260 ng/mL was associated with

---

[2] Hogan D, Fox P, Jacob K, et al. Analysis of the feline arterial thromboembolism: clopidogrel vs. aspirin trial (FAT CAT). In: proceedings of the ACVIM forum; June 13-15, 2013; Seattle, WA (abstract).

thromboprophylaxis in a wide variety of scenarios (including orthopedic surgery and atrial fibrillation).[11-15]

In humans, both prothrombin time (PT) and activated partial thromboplastin time (aPTT) are prolonged at therapeutic plasma drug concentrations of RVX[16,17] The assays' ability to detect low drug concentration, and prolongation across a range of concentrations vary widely due to differential sensitivity of the reagents used to trigger coagulation in the assay mixtures. The PT is generally a more accurate measure of RVX anticoagulant action than aPTT, and PT sensitivity and linearity to detect RVX has been further enhanced through the use of a diluted thromboplastin reagent.[18] The dilute PT assay (dPT) may create an *in vitro* environment that is more physiologic than the standard PT.[18,19] The effect of RVX on coagulation parameters has not been studied in cats; however, a previous *in vitro* study showed that RVX delayed fibrin endpoint formation in plasma from healthy cats as it does in other species.[3] The specific FXa inhibitory activity (aXa) of RVX can be measured directly using functional assays configured with chromogenic FXa substrates.[17-19]

The primary goal of this study was to evaluate the pharmacokinetic and pharmacodynamic profile of single and multiple oral doses of RVX in healthy adult cats. In addition, adverse effects associated with both short- and long-term administration of RVX were evaluated. We hypothesized that administration of oral RVX to healthy adult

---

[3] Brainard BM, Cathcart CJ, Dixon AC, et al. In vitro effects of rivaroxaban on feline coagulation indices. In: proceedings of the ACVIM forum; June 15-18, 2011; Denver, CO (abstract).

cats would result in plasma drug concentrations within the target range reported for thromboprophylaxis in humans, result in dose-dependent changes in aXa, dPT, and aPTT, and be well-tolerated and safe for use in cats.

Materials and Methods

Subjects. Six purpose-bred adult domestic shorthair cats (3 males, 3 females) were housed in accordance with AAALAC guidelines. Weights ranged from 2.3 to 6 kg. The research protocol was approved by the University of Georgia IACUC (A2010 11-591-Y1-A0). Cats were deemed healthy based upon results of physical examination, complete blood count (CBC), serum biochemistry analysis, coagulation profile, urinalysis, total serum thyroxine concentration, indirect arterial blood pressure measurement using a Doppler ultrasonic flow detector[4], and echocardiography. Cats were evaluated at least twice daily during drug administration for adverse events including bleeding, anorexia, weight loss, and other gastrointestinal signs.

Commercially available 10 mg RVX tablets[5] were halved or quartered with a pill splitter and then weighed to determine the dosage, making the assumption that RVX was evenly distributed throughout the tablet, and that the 10 mg FDA approved product actually yielded 10 mg of active RVX per tablet. When multiple doses were administered, each individual cat would receive subsequent doses from the remainder of the single tablet that was initially split. To obtain 1.25 mg doses, RVX was made into a tablet triturate. RVX 10 mg tablets including the coating, were crushed to a fine powder and sieved to obtain a uniform particle size. Lactose was added as a diluent and 35% ethanol used to

---

[4] Parks Medical Electronic Inc, Aloha, Oregon
[5] Xarelto 10mg tablets, Bayer HealthCare AG, Leverkussen, Germany

moisten the combined powders. The tablets were created using manual pressure to force moistened tablet material from a calibrated die set. The formed tablets were allowed to dry before packaging for dispensing. Sample tablet triturates from this lot were also submitted for analysis of active drug content.[6]

Single oral dose. Using a prospective, randomized, cross-over design, cats were assigned to receive a single oral dose of 1.25 mg, 2.5 mg, or 5 mg RVX. A minimum washout period of 2 weeks occurred between different dose administrations. The day prior to drug administration, cats were sedated using a combination of dexmedetomidine[7] (5 mcg/kg IV) and butorphanol[8] (0.2 mg/kg IV), and jugular venous catheters[9] were placed percutaneously using a modified Seldinger technique. Catheters were flushed with 50% dextrose[10] when not in use for sampling, and flushed with 0.9% saline[11] during sampling times. All blood sampling was performed using the jugular catheters unless otherwise noted. A waste sample of 3 mL was withdrawn from the catheter (priming volume of 0.7 mL), before study samples were collected.

Six cats were given a single oral dose of 1.25 mg RVX, and samples for analysis of coagulation function (dPT, aPTT, aXa activity)[20] were obtained at 0, 1, 3, 12 and 24 hours following oral dosing. Six cats were given a single oral dose of 2.5 mg RVX, and coagulation samples were obtained at 0, 1, 3, 8 and 24 hours. Initial data from the 1.25mg RVX group showed a drug effect at 3 hours and little effect at 12 hours, so sampling times were altered in order to obtain an 8 hour time point. Due to concerns about

---

[6] Compounders International Analytical Laboratory, Castle Rock, CO
[7] Dexdomitor, Zoetis, Florham Park, NJ
[8] Torbutrol 10mg/ml, Zoetis, Florham Park, NJ
[9] 20 ga. 13 cm single-lumen catheter, Arrow Teleflex International, Raleigh, NC
[10] Dextrose 0.5g/mL, Hospira, Lake Forest, IL
[11] 0.9% Sodium Chloride 1000mL, Hospira Inc, Lake Forest, IL

excessive phlebotomy, the 12 hour time point was not collected. Blood samples were also obtained at 0, 5, 10, 15, 30, 45, 50, 90, 120, 180, 240, and 480 minutes following the 2.5mg dose administration for analysis of plasma RVX concentration. Three cats were given a single oral dose of 5 mg RVX and samples for coagulation analysis were obtained at 0, 3, 8 and 24 hours following dosing. The 1 hour time point was not sampled in these cats to decrease the amount of blood removed from each cat and based on evidence of peak effect occurring at 3 hours post-administration. Blood samples for coagulation analyses at each RVX dose and for each arm of the study were collected into tubes containing 3.2% sodium citrate[k] for a final citrate:blood ratio of 1:9. Blood for analysis of RVX concentration was collected into tubes containing EDTA.[12] Following collection, the tubes were centrifuged at 2,700 x g for 10 minutes, and the supernatant was frozen at -80° C until batch analysis was performed at the Comparative Coagulation Section of the Animal Health Diagnostic Center at Cornell University (coagulation analysis) and the Auburn University Specialized Pharmaceutical and Experimental Center for Translational Research (RVX plasma concentration). Criteria for evaluation of aXa values were based upon extrapolations from human investigations of thrombosis and LMWH dosing, suggesting that a therapeutic window for thromboprophylaxis existed between aXa values of 0.5 and 1.0 IU/dL.[21]

3-day oral dosing: Six cats were given 1.25 mg RVX PO as a tablet triturate every 12 hours for 3 days and coagulation samples were obtained as described above at baseline (time 0) and 1, 3, and 12 hours following oral dosing on day 1. Coagulation samples were obtained following the final oral dose on day 3 at 0, 3, and 12 hours.

---

[12] BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ

7-day oral dosing: Six cats were given 2.5 mg RVX PO every 24 hours for 7 days and samples for coagulation analysis were obtained prior to dosing (time 0), and at 1, 3, and 8 hours on days 1 and 7. Coagulation samples were obtained prior to administration of RVX at time 0 and 12 hours following the dose on days 2 and 3. On days 5 and 6, blood was collected for coagulation analysis only at 12 hours after dose administration, due to concerns for the development of iatrogenic anemia from repeated blood sampling. A CBC, serum biochemistry, and urinalysis were performed on cats in this group on day 7 and day 14 (i.e., one week after RVX was discontinued).

28-day oral dosing: Based on analysis of aXa activity from the previous dosing regimens, a dose of 1.25 mg RVX was studied under conditions of longer term, once daily dosing. Six cats were given 1.25 mg RVX PO every 24 hours for 28 consecutive days. Coagulation samples were obtained by jugular venipuncture using 20-ga needles and syringes due to technical difficulties with long-term maintenance of indwelling jugular catheter patency. Samples were collected prior to dosing (time 0), and at 1, 3, and 8 hours following the oral dose on day 1 and day 28. On days 7, 14, and 21, samples were collected at the 0-hr time point, immediately prior to administration of the daily dose. Blood samples for CBC, serum biochemistry, and urinalysis were obtained on day 28. Two weeks after RVX was discontinued, blood samples were obtained for a CBC, serum biochemistry, urinalysis, and a coagulation profile.

Clotting time tests (aPTT, dPT) and aXa Assays: All assays were performed with an automated coagulation instrument[13] using mechanical or spectrophotometric endpoint

---

[13] STA Compact, Diagnostica Stago, Parsippany, NJ

detection. The aPTT reagent[14] contained an ellagic acid activator and soy phospholipid and dPT was performed using a rabbit brain thromboplastin reagent[15] prediluted 1:10 in an imidazole buffer containing 0.025 M calcium chloride. The aXa concentrations (IU/mL) were measured using a commercial kit[16] calibrated against a human plasma standard containing low molecular weight heparin (LMWH),[17] as previously described.[20] The manufacturer subsequently developed an RVX calibration standard[18] for the aXa assay and banked plasma samples from the single dose studies were also assayed to determine aXa concentrations in ng/mL RVX against this standard.

Pharmacokinetic data: For analysis of serum RVX concentration, a high-pressure liquid chromatography tandem mass spectrometric (LC-MS/MS) analysis was developed. Briefly, blank cat plasma was used for preparation of standard solutions. A serial dilution of a RVX standard[19] solution was prepared to concentrations of 1310, 655, 327.5, 163.75, 81.88, 40.94, 20.47, 10.23, 5.12, and 2.56 ng/mL. Quality control samples were prepared independently at concentrations of 655, 40.94, and 2.56 ng/mL.

A 30 μL aliquot of sample was combined with 30 μL of internal standard (Linezolid[19], 500 ng/mL in acetonitrile[20]) solution. 90 μL of ice-cold acetonitrile was added to perform deproteinization and the mixture was then vortex-mixed for 30 sec and centrifuged for 20 min at 14,000 x g. An aliquot of 50 μL of the supernatant was transferred to an auto-sampler vial and injected on-column (1 μL) for LC-MS/MS

---

[14] Dade Actin FS, Dade Behring, Newark, DE
[15] Thromboplastin LI, Helena Diagnostics, Beaumont, TX
[16] STA-Liquid Anti-Xa, Diagnostica Stago, Parsippany, NJ
[17] STA-Multi Hep Calibrator, Diagnostica Stago, Parsippany, NJ
[18] STA-Rivaroxaban Calibrator, Diagnostica Stago, Parsippany, NJ
[19] Selleck Chemicals, LLC, Houston, TX
[20] Sigma-Aldrich, St. Louis MO

analysis.[21] The mobile phase consisted of 0.1% formic acid and acetonitrile. The samples

were separated on a column[22] using a gradient from 20% acetonitrile to 100% over 1 min,

and then maintained at 100% for 30 sec. The samples were introduced into the mass

spectrometer with a flow rate of 0.4 mL/min using a jet-stream electrospray ionization

source.[23] Nitrogen gas was used as the dry (10 L/min at 350 ºC), nebulizer (45 psi), and

collision gas. Capillary voltage was set at 4000 V. Mass spectra were acquired in positive-

ion mode, and mass transitions were monitored using multiple-reaction monitoring. The

transitions for each analyte are listed in Table 4.1.

Table 5.1: Mass transitions used for quantification and qualification in LC-MS/MS
analysis of rivaroxaban and the standard linezolid for analysis of plasma rivaroxaban
concentrations in cats.

Compound	Type	Transition	Fragmentor	Collision Energy
Rivaroxaban	Quantifier ion	436.1 – 231.1	110	18
	Qualifier ion	436.1 – 145.0	110	27
Linezolid	Quantifier ion	338.2 – 296.2	125	14
	Qualifier ion	338.2 – 235.0	125	17

The method was linear from RVX concentrations of 2.56 to 1310 ng/mL, with the limit

of quantification (LOQ) of 0.5 pg on column and accuracies > 90%, and variation < 15%.

---

[21] Agilent 1290 UHPLC system coupled Agilent 6460 Triple Quad mass spectrometer,
Agilent Technologies, Santa Clara, CA
[22] Agilent ZORBAX SB-C18 column (2.1 x 50 mm, 1.8 µm), Agilent Technologies,
Santa Clara, CA

[23] Agilent Jet stream Electrospray Ionization source, Agilent Technologies, Santa Clara,
CA

Pharmacokinetic analysis: Pharmacokinetic (PK) parameters including maximum observed concentration (C_{max}), time of C_{max} (t_{max}), terminal elimination rate (β), terminal half-life ($t_{1/2}$), apparent volume of distribution/bioavailability (Vd/F), total systemic clearance/bioavailability (CL/F) and area under the plasma drug concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) were estimated from a noncompartmental analysis using the nonlinear regression analysis program.[24] AUC was estimated using the log-trapezoidal method and extrapolating exposure from last measured concentration to infinity. The oral bioavailability (F) was not determined directly, therefore the Vd and CL are shown as a function of bioavailability.

Statistical Methods

Descriptive statistics were generated using a commercially available statistical software program.[25] Data were tested for normality using the Kolmogorov-Smirnov test. Normally distributed data are reported as the mean ± one standard deviation, non-parametric data are reported as median (minimum value - maximum value). Sequential values were compared using a one-way ANOVA for repeated measures. The Pearson product-moment correlation coefficient was generated to compare mg/kg dosage versus coagulation parameters. The difference in the aXa values among the different drug doses was evaluated by pairwise multiple comparison procedures (Holm-Sidak method) because the data was not normally distributed. Some single comparisons for the multi-day dosing protocols were accomplished using a paired t-test. *P* values < 0.05 were considered significant. Corrections (Bonferroni method) for multiple comparisons were performed

---

[24] WinNonlin Professional version 5.3, Pharsight Corp., Mountain View, CA
[25] Prism v.6, GraphPad Software Inc., La Jolla, CA

where appropriate. The measured (LC-MS/MS) and estimated plasma RVX

concentrations were compared using both Pearson product-moment correlation and Bland-

Altman analysis.

Results

In the single-dose studies of RVX at 1.25 mg, 2.5 mg and 5 mg, the average dosage of

RVX per cat was 0.35 ± 0.1 mg/kg, 0.73 ± 0.2 mg/kg, and 1.3 ± 0.4 mg/kg, respectively.

The drug was well tolerated without evidence of hemorrhage or gastrointestinal upset.

Sample tablets were evaluated from the lot used for this study and the potency assay

results for the tablet tritruates showed an average of 1.151 mg active ingredient per tablet

(92.1% of the expected potency), and this is taken into account in the calculations for this

dose. Each cat was evaluated with a CBC, serum blood chemistry, and urinalysis after

single oral dosing of 1.25mg, 2.5mg, and 5mg RVX, after twice daily dosing of 1.25mg

RVX, and after multiple daily dosing of RVX. All values fell within normal institutional

reference ranges for each time point obtained.

 After single oral doses of 1.25 mg, 2.5 mg and 5 mg, we found dose-related

increases in aXa (Figure 5.1).

Figure 5.1: The mean anti-Xa activity (IU/mL) after rivaroxaban administration plotted with one standard deviation for single oral rivaroxaban doses of 1.25mg (triangle), 2.5mg (circle) and 5mg (square) to 6 healthy adult cats. There was a dose dependent increase in anti-Xa activity with peak activity occurring at 3 hours post-administration. Anti-Xa activity was gone by 12 hours after dosing of 1.25mg rivaroxaban but residual activity was noted after 24 hours in either the 2.5mg and 5mg rivaroxaban doses.

Anti-Xa activity in feline blood
following a single oral dose of rivaroxaban
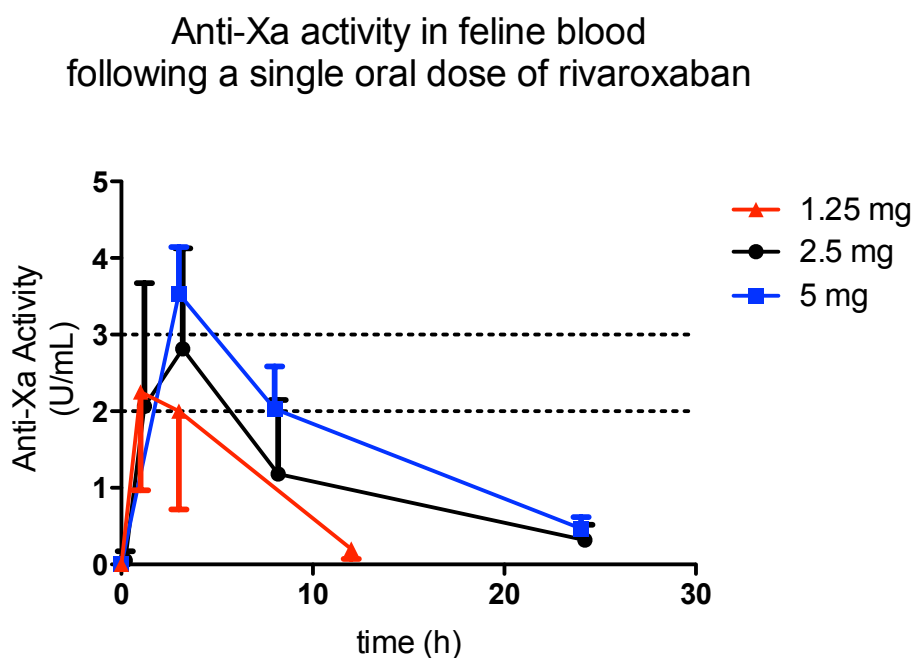
RVX at the two lower dosages (1.25 and 2.5 mg) resulted in aXa at or above 0.5 IU/mL at 1 hour post-administration, with peak aXa attained at 3 hours. The peak aXa for the 1.25, 2.5, and 5 mg doses were 2.0 ± 1.3, 2.8 ± 1.3, and 3.5 ± 0.6 IU/mL, respectively. The 3-hour aXa for the 5 mg dose was significantly greater than the 3-hour aXa for the

1.25mg dose ($P = 0.011$). While residual aXa activities of 0.3 ± 0.2 and 0.47 ± 0.2 IU/mL

($P = 0.303$) were detected at 24 hours following a single dose of 2.5 and 5 mg,

respectively, the cats that received 1.25 mg had aXa values that fell to 0.2 ± 0.1 IU/mL

by 12 hours after the single dose. The aXa activities were not measured beyond the 12-

hour time point in this group. There were no signs of abnormal hemorrhage in any cat,

even those receiving RVX at 5-mg dosage when aXa values rose beyond three times the

targeted aXa range (extrapolated from human data).

Single oral dose RVX produced a dose-dependent prolongation of dPT (Figure

5.2).

Figure 5.2: Dilute prothrombin time (dPT) plotted against time for single oral RVX

doses at 1.25mg (triangle), 2.5mg (circle), and 5mg (square) administered to 6 healthy

adult cats. The dotted line indicates the feline normal dPT value from pooled sera of

healthy adult cats. There was a dose-dependent prolongation of dPT noted with peak

prolongation occurring at 3 hours. The dPT values at the 3-hour time point were not

significantly different among the 3 doses.

Dilute prothrombin time in feline blood following once daily dosing of rivaroxaban
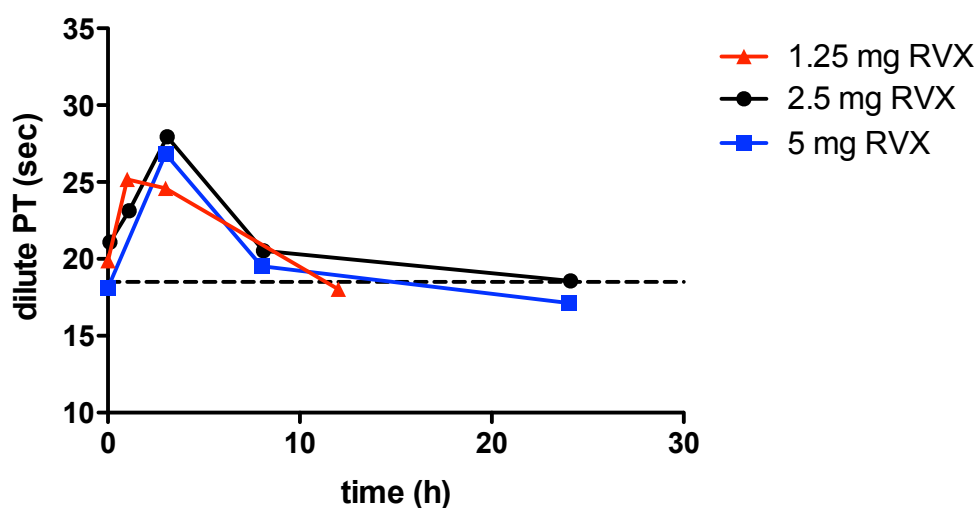
Peak prolongation over a baseline value of 20.0 ± 3.1 sec was seen at 3 hours after oral

administration, to 24.6 ± 5.1 sec for the 1.25 mg dose, 28.0 ± 6.0 sec for the 2.5 mg dose,

and to 26.8 ± 7.3 sec for the 5 mg dose. The dPT values at the 3-hour time point were not

significantly different among doses ($P = 0.608$), but the prolongation from baseline was

significant in the 1.25-mg dose group ($P = 0.007$). The prolongation was not significant in

either the 2.5 mg dose group (*P*= 0.03 but did not reach critical value after correction for multiple comparisons) or the 5 mg dose group (*P*= 0.064). Comparisons with baseline dPT revealed no significant differences for 12-hour dPT (1.25mg dose group) or the 24-hour dPT (2.5 and 5mg dose groups). aPTT was not measured in the 1.25 mg dose group, but did not show significant changes from baseline at the 3-hour time point in either the 2.5 mg or the 5 mg dose group (*P* = 0.185 and *P* = 0.053, respectively, Figure 4.3).

Figure 5.3: Activated partial thromboplastin time (aPTT) plotted against time for single oral RVX doses at 2.5mg (circle) and 5mg (square). The dotted line indicates the feline normal aPTT value from pooled sera of healthy cats. aPTT did not show significant change from baseline at the 3 hour time point in either dose group.

Activated partial thromboplastin time in feline blood following once daily dosing of rivaroxaban
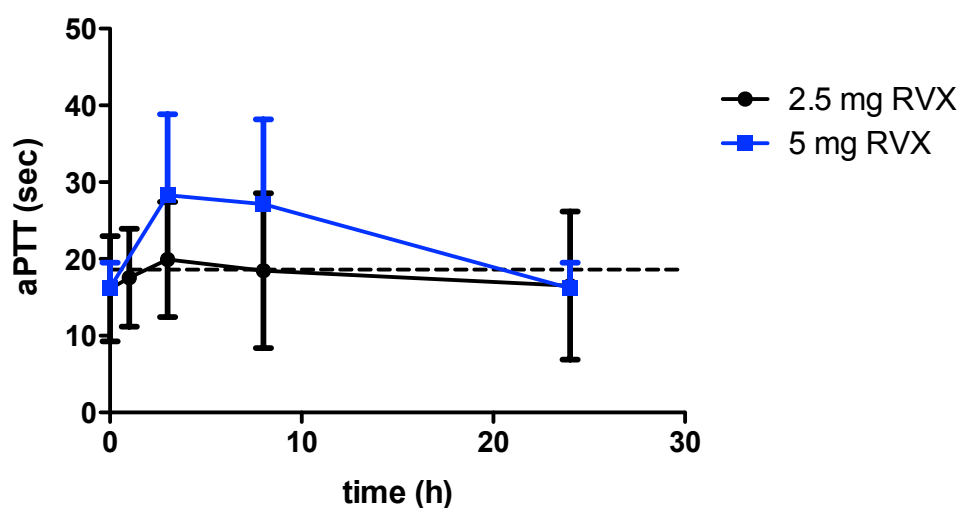
Slight differences in aXa concentrations were seen between cats given a single 1.25 mg RVX dose compared with values obtained from cats administered 1.25mg RVX every 12 hours for 3 days. Twice daily dosage resulted in peak aXa of 2.3 ± 1.3 IU/mL at 1 hour following oral dosing, with 3-hour aXa of 2.0 ± 1.3 IU/mL. There was no apparent cumulative effect of multiple dosing on peak aXa, with values of 1.8 ± 0.5 IU/mL at 1 hour and values of 1.7 ± 0.7 IU/mL at 3 hours after dosing on treatment day 3. At 12 hours following the final dose on day 3, the aXa was 0.3 ± 0.08 IU/mL, which was higher than aXa at 12 hours following the first oral dose (0.2 ± 0.13 mg/dL, $P = 0.013$). In this group, the dPT values followed similar patterns as the single oral dose group (data not shown).

The effects of a 2.5 mg RVX oral dose given once daily over a period of 7 days were also evaluated (Figure 5.4).

Figure 5.4: The mean anti-Xa activity (IU/mL) after 2.5mg oral RVX administration for 7 days (circle) plotted with two standard deviations (bars). There was no difference in anti-Xa activity at the 3 hour time point on day 1 versus day 7 indicating daily administration did not result in a cumulative anti-Xa activity effect.

Anti-Xa activity in feline blood following once daily dosing of rivaroxaban (2.5 mg) for 7 days
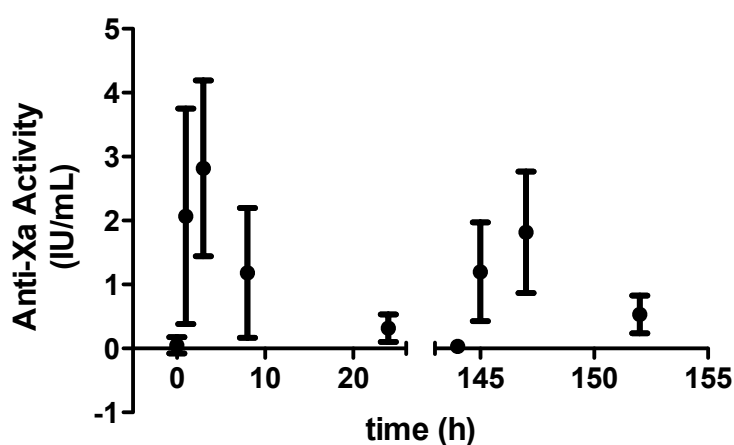
Anti-Xa activity at 3 hours following the first dose was 2.8 ± 1.3 IU/mL and the aXa activity at 3 hours following the last dose (day 7, after 6 days of therapy), was 1.8 ± 0.9 IU/mL ($P = 0.168$). 24 hours after the first dose, aXa activity was 0.3 ± 0.2 IU/mL, and 24 hours after the day 6 dose, aXa activity was 0.03 ± 0.05 IU/mL ($P = 0.010$). Anti-Xa values 12 hours following the administered RVX doses on days 5 and 6 were 0.1 ± 0.1 and 0.1 ± 0.08 IU/mL, respectively ($P = 1.00$).

In cats that received RVX at 1.25 mg PO every 24 hours for 28 days, the aXa activity was 1.8 ± 0.9 IU/mL at three hours following the final oral dose on day 28. aXa activity immediately prior to the oral dose on days 7, 14, 21, and 28 did not exceed 0.1

IU/mL in any cat (Figure 4.5), however this value on day 21 was 0.08 ± 0.04 IU/mL, and

was significantly higher than that measured on day 0 and day 42 (both 0 ± 0 IU/mL, $P =$

0.004).

Figure 5.5: The mean anti-Xa activity (IU/mL) after 1.25mg oral RVX administration for

28 days (circle) plotted with two standard deviations (bars). The last day of

administration (day 28) is shown with mean anti-Xa activity at the 0, 1, 3, and 8 hours

time point. Peak anti-Xa activity was noted at the 3 hour time point and this peak was

not significantly different from the 3 hour time point taken on day 1.

**Anti-Xa activity in feline blood following
multiple oral doses of 1.25 mg rivaroxaban**
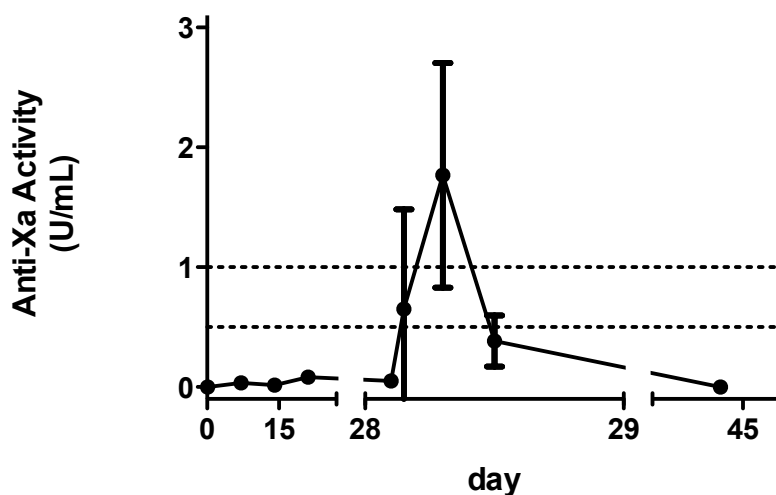
In the cats receiving RVX at 1.25 mg PO every 24 hours for 28 days, the dPT

measured at three hours following the final oral dose on day 28 was significantly

prolonged from baseline to 24.4 ± 4.0 sec ($P= 0.0008$). The dPT measured on day 0 prior

to any drug administration was 20.2 ± 0.8 sec, and was not different from that measured

on day 28 at time 0 (*P*= 0.434). There were minimal differences between other nadir

sampling times, with the exception of the dPT measured on day 28 (19.2 ± 0.3 sec) and

day 42 (18.1 ± 0.7 sec, *P* = 0.03). aPTT did not change for the duration of the dosing for

any of the nadir values (*P* = 0.173), and did not change at any time on day 28 following

the final oral dose (*P* = 0.110).

Based on the individual cat weights, the dosage was calculated and compared to

the peak (3-hour) aXa concentrations using the Pearson product moment correlation,

which showed a significant association and a Pearson's r =0.66 (*P* <0.001, Figure 4.6).

Figure 5.6: Linear correlation between the mg/kg RVX dose and plasma anti-Xa activity

using the Pearson product moment correlation. There was a significant association

between the two variables with increasing RVX dose correlating with increasing anti-Xa

activity.
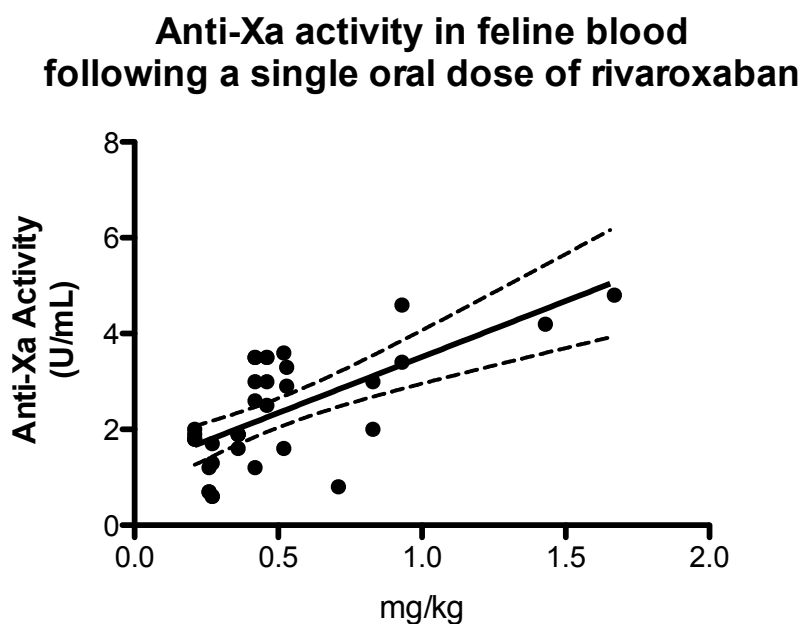
Correlation between dosage and dPT times was also significant (r=0.53, P <0.001), however, there was no correlation between dosage and aPTT (r=0.26, P= 0.32).

When evaluating the correlation between aXa activities obtained across all trials with the measured coagulation variables, there was a significant but fair correlation between aXa activity and dPT (r=0.6, P < 0.001), but not between aXa activity and aPTT (r=0.16, P = 0.19).

Pharmacokinetics

Pharmacokinetic data are summarized in Table 5.2.

Table 5.2: Pharmacokinetic data for 5 cats given an oral dose of 2.5 mg of rivaroxaban (RVX). t_{max} : time to maximal plasma RVX concentration, C_{max}: maximal plasma RVX concentration, β: terminal elimination rate, $t_{1/2}$: terminal half-life, Vd/F: apparent volume of distribution/bioavailability, CL/F: total systemic clearance/bioavailability, and $AUC_{0-\infty}$: area under the plasma drug concentration-time curve from time 0 to infinity.

Parameters (Unit)	Dose (2.5mg)					Mean	Standard Deviation
	Cat A	Cat B	Cat C	Cat D	Cat E		
Dose (mg/kg)	0.521	0.417	0.725	0.833	0.532		
t_{max} (hr)	2	2	4	1.5	3	2.5	1.0
C_{max} (ng/mL)	82.9	250	413	412	196	271	143
β (hr^{-1})	0.0574	0.0764	0.0996	0.142	0.147	0.105	0.040
$t_{1/2\beta}$ (hr)	12.1	9.07	6.96	4.88	4.72	7.55	3.10
$AUC_{0-\infty}$ (hr·ng/kg·mL)	1,000	194,000	241,000	132,000	64,400	140,500	76,900
Vd/F (mL)	7670	1690	1810	2660	3370	3440	2461
CL/F (mL/hr)	7.34	2.15	3.01	6.29	8.26	5.41	2.69
AUCinf/D (hr/mL)	0.136	0.466	0.332	0.159	0.121	0.243	0.151
Vd/F/D (kg·mL/mg)	14700	4040	2500	3190	6340	6154	4992
CL/F/D (kg·mL/hr·mg)	14.1	5.15	4.15	7.55	15.5	9.29	5.20

Due to incomplete sampling of one of six cats (terminal phase was not adequately captured to accurately estimate PK parameters), one cat was excluded from the summary analysis. Plasma RVX concentrations for the single dose patients (n=5) were analyzed using the dosage by body weight. The actual dosage ranged from 0.42 to 0.83 mg/kg, and C_{max} ranged from 82.92 to 413 ng/mL. Dosage was significantly correlated to C_{max} (r= 0.89, $P = 0.02$). C_{max} was reached between 90-240 min following the administration of

the oral dose. Correlation between the RVX plasma concentrations measured by LC-

MS/MS and measured aXa using the LMWH calibrator showed a strong relationship

(r=0.86, *P*<0.0001, Figure 5.7).

Figure 5.7: Pearson product moment correlation showing the linear correlation between

plasma RVX concentration (ng/mL) and inhibition of anti-Xa concentration (IU/mL).

The dashed lines represent the targeted plasma concentration for effective

thromboprophylaxis in people of 140-240 ng/mL. The anti-Xa concentrations associated

with targeted plasma RVX concentrations (2.6-4 IU/mL) were higher than the

extrapolated target anti-Xa values established for people taking LMWH of 0.5-1.0

IU/mL.
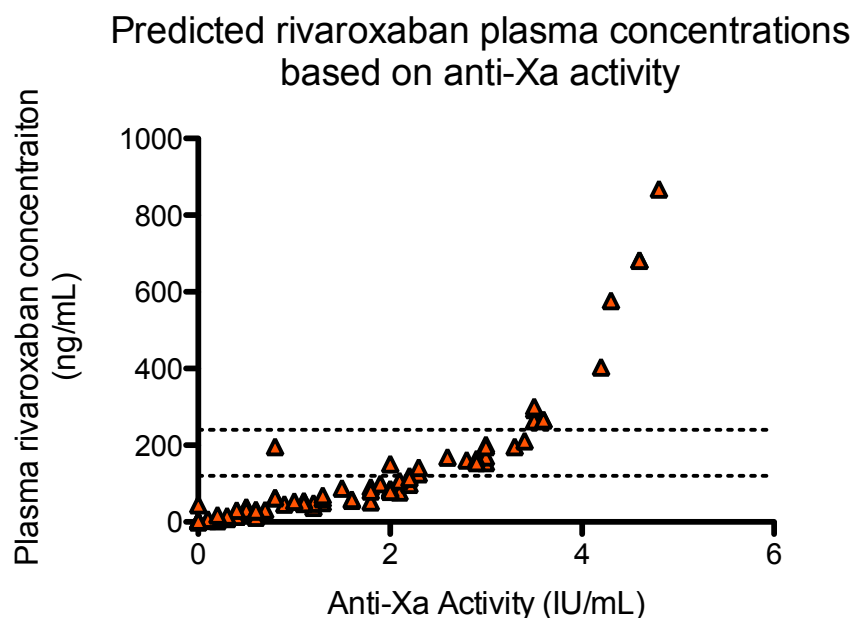
The correlation between LC-MS/MS-measured RVX concentrations and RVX measured in the RVX-calibrated aXa assay was excellent (r=0.985, *P*<0.0001). To further compare these two methods, a Bland-Altman analysis was performed, using the LC-MS/MS-derived measures as a gold standard. The Bland-Altman analysis shows a constant bias of -8.518 ng/mL, with a standard deviation of bias of 55.5 ng/mL. 95% limits of agreement on this plot were -117.3 to 100.3, and appeared to be greatest on plasma concentrations greater than 600 ng/mL (Figure 4.8). Excluding values above 600 ng/mL measured with either method yielded a bias of 6.82 ±19.1, with 95% limits of agreement from -30.6 to 44.25 ng/mL.

Figure 5.8: Bland-Altman plot demonstrating the agreement between HPLC-measured RVX concentrations and RVX measured in the RVX-calibrated aXa assay (Stago method). There was excellent agreement with this method between anti-Xa concentrations and RVX concentration.

Bland-Altman comparison of RVX concentration determined by HPLC to those determined by the Stago protocol
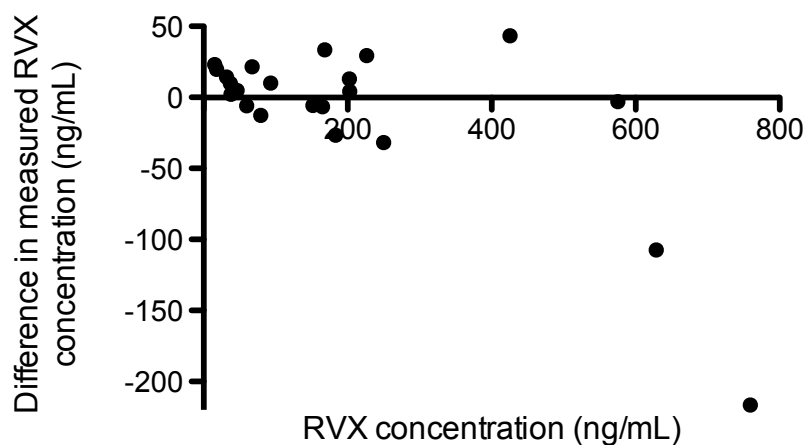
Discussion

Oral RVX was well tolerated in healthy adult cats at a number of different doses and dosing regimens. We found significant correlations between RVX dosage and aXa, and significant differences in mean peak aXa in response to 1.25-mg, 2.5-mg, and 5-mg doses. Clotting times were prolonged from baseline at the time of peak plasma RVX, with a direct correlation between dPT and aXa. The dPT was prolonged in concert with RVX concentration, and in animals that reached the human target plasma concentration of RVX (i.e. above 140 ng/mL[9]), dPT was significantly prolonged from the baseline at 3 hours following dose administration. However, the prolongation of dPT from baseline was only significant in the 1.25mg dose group. This could be due to insensitivity of dPT to distinguish between different doses, but is more likely a result of the small sample size. In cats that achieved an aXa of 3 IU/mL, the average dPT prolongation was 1.35 times the baseline, and in two cats that achieved an aXa of 3.6 IU/mL, the average dPT prolongation was 1.8 times the baseline value. When plasma RVX concentrations > 140 ng/mL were obtained in the study cats (i.e., the human target therapeutic concentration), the corresponding aXa concentrations were higher (2.6-4 IU/mL) than the initially targeted aXa range of 0.5 to 1.0 IU/mL, which was derived from the values used for LMWH thromboprophylaxis in people.[21]

All RVX doses resulted in aXa within the target range for human thromboprophylaxis, although at lower doses the target activities were not sustained. Measurement of aXa has been used in human clinical studies of thromboprophylaxis because it is a direct assessment of drug concentration in plasma based on a relevant

biologic effect, and can be studied regardless of the type of heparin that is used.[22] Target concentrations for aXa have been developed for the prevention and treatment of various thrombotic syndromes in people. These concentrations have been extrapolated for use in both dogs and cats, although outcome-based recommendations for optimal aXa have not been established yet in these species.[23]

Similar to people, peak plasma RVX concentration and aXa activity after oral administration occurred at 3 hours in cats. In the 2.5 mg and 5 mg dose groups, anticoagulant effects had returned to baseline by 24 hours. The duration of action of 1.25 mg RVX given orally does not appear to extend beyond 12 hours. In people, RVX administered twice daily for the treatment of acute coronary syndromes (ATLAS ACS2-TIMI 51) decreased ischemic events but increased adverse events such as major bleeding and intracranial hemorrhage.[24] Even with once-daily dosing, which results in a portion of the day with low aXa activity, human studies have shown positive outcomes in thrombophilic conditions[25] like deep vein thrombosis (EINSTEIN-DVT)[12] and non-valvular atrial fibrillation (ROCKET-AF).[11] Furthermore, in a venous stasis model of healthy cats administered parenteral enoxaparin, an anti-thrombotic effect was seen despite aXa concentrations that did not achieve targeted ranges.[26] The pharmacodynamics of RVX in once-daily human trials mirrors the pharmacodynamics seen in healthy cats in the current study.

Cats were administered 1.25 mg RVX orally once-daily for 28 days to determine whether progressive anticoagulant effect due to delayed hepatic processing had occurred. Even though some RVX metabolism occurs through the p-glycoprotein system, no residual drug effect was seen in the pharmacodynamic profile of the drug. It is possible

that co-administration with inhibitors of p-glycoprotein could raise plasma concentrations of RVX and prolong or intensify anticoagulant effects. The possibility also exists that co-administration of RVX with inhibitors of the P450 system could result in increased drug excretion and clearance and alter the therapeutic effect.[27] In some individuals, the aXa was slightly less on the final treatment day than on the initial day, which may reflect induction of enzyme systems and more rapid drug clearance, although this hypothesis was not specifically tested. There did not appear to be adverse systemic effects of either the short-term or long-term dosing regimens tested in this study, and no abnormalities were detected on CBC, serum biochemical analysis, and urinalysis testing during this time frame. As in people, the pharmacokinetics and pharmacodynamics of RVX do not appear to be affected by gender, but this hypothesis was also not specifically tested and the sample size was small (3 male, 3 female).[28] Several risk factors that have been identified as conferring an increased risk of bleeding in human beings receiving RVX include hepatic disease, renal disease, malignancy, thrombocytopenia, hypertension, and anemia.[29] The cats in this study were routinely tested with bloodwork consisting of CBC and serum biochemistry analyses. No risk factors were identified; however, the PCV did decrease with repeated blood draws. These values remained within normal limits but there is a possibility that decreasing PCV might have affected RVX pharmacodynamics.

In addition to aPTT, dPT was used to assess anticoagulant activity in this study based on test strategies for RVX monitoring in people.[30] We found a statistically significant correlation between RVX dosage, aXa, and dPT, while there was no correlation between dosage and aPTT. Measurements of aXa and dPT may therefore prove useful in future clinical trials to develop RVX dosage regimens for cats but the use

of dPT requires additional study. The commercially available kit to measure plasma RVX concentration using a chromogenic assay had excellent correlation with plasma RVX concentrations determined by LC-MS/MS, and is accurate throughout a wide concentration range.[17] While based on measurements of aXa, this assay requires a specific RVX calibrator that increases its cost and limits it use to RVX monitoring. The optimal approach to monitor RVX anticoagulant action, and ultimately develop safe and effective regimens for thromboprophylaxis in cats with HCM will require clinical trials. The dPT is a relatively inexpensive clotting time test that demonstrated predictable, linear, dose-dependent prolongation and may prove useful for RVX monitoring. Additional studies are needed, however, to confirm that the dose-dependent prolongations of dPT seen in healthy cats would similarly occur in cats with hypercoagulability due to disease. In human medicine, RVX is typically administered at a fixed dosage based on indication, with no routine monitoring of coagulation parameters. Coagulation testing is reserved for patients with decreased renal function, other risks for overdose and/or overt hemorrhagic complications.[18] Ultimately, clinical use of RVX in cats may follow this practice.

This study has a number of limitations. First, the small number of cats that were studied may not be representative of the larger feline population. In addition, RVX was only tested in healthy cats. RVX pharmacokinetic and pharmacodynamic profiles have been described in rat, rabbit, dog, and human models.[16,31-34] In rats, dogs, and humans, RVX is cleared primarily unchanged through renal and fecal-biliary routes[35] while smaller amounts are metabolized through hydrolysis[35] and cytochrome p450 pathways.[36,37] The pharmacodynamics of RVX may differ based upon co-existing hepatic and renal disease in cats and these populations should be further tested. Fixed amounts of RVX were given

without dose adjustments for weight. While this may be an adequate representation of the clinical usage of the drug, the lack of a standard dosage may have resulted in relatively large standard deviations of anticoagulant effects. Because many cats are similar in body weight, this may not be a major limitation, but specific weight-based dose adjustment (e.g., using a suspension of drug) was not attempted in this study. An attempt to manufacture a flavored drug suspension resulted in significant adverse reactions (specifically, hypersalivation) to the taste of a number of different flavors.

In this study, aXa was not maintained in the human therapeutic range for the duration of a 12-hour or 24-hour dosing interval. Once-daily dosing of RVX, however, has proven to be efficacious for certain indications in people, such as prevention of venous thrombosis after orthopedic procedures.[16,38-41] In contrast, twice daily dosing of RVX is recommended for people with acute coronary syndromes.[24] The pharmacodynamics of RVX in cats mirrors those obtained in people, however, the true efficacy of RVX therapy in cats cannot be assessed without specific outcome-based measures. Pharmacodynamic testing of RVX administered twice daily at a higher dose (e.g., 2.5 mg) is therefore warranted since twice-daily administration might offer more effective thromboprophylaxis in cats with hypercoagulable conditions that are at high risk of thrombosis. Alternatively, lower doses of RVX may be indicated to prevent adverse bleeding events if combined with anti-platelet therapy, but this was not specifically evaluated in this study.

Oral RVX was safe and well tolerated in healthy cats. Oral administration resulted in predictable plasma drug concentration within the target range for effective thromboprophylaxis in human beings and caused dose-dependent prolongation of dPT. Oral administration of RVX also caused a more reliable dose-dependent increase in aXa

concentrations. Our study suggests that RVX holds promise for thromboprophylaxis in cats with heart disease and that clinical trials in these patients are warranted.

References

1.   Paige CF, Abbott JA, Elvinger F, et al. Prevalence of cardiomyopathy in apparently healthy cats. *J Am Vet Med Assoc* 2009;234:1398-1403.

2.   Peterson EN, Moise NS, Brown CA, et al. Heterogeneity of hypertrophy in feline hypertrophic heart disease. *J Vet Intern Med* 1993;7:183-189.

3.   Rush JE, Freeman LM, Fenollosa NK, et al. Population and survival characteristics of cats with hypertrophic cardiomyopathy: 260 cases (1990-1999). *J Am Vet Med Assoc* 2002;220:202-207.

4.   Baker LD, Birk P. Removal of aortic thrombi in the cat. *Mod Vet Pract* 1974;55:303.

5.   Smith SA, Tobias AH, Jacob KA, et al. Arterial thromboembolism in cats: acute crisis in 127 cases (1992-2001) and long-term management with low-dose aspirin in 24 cases. *J Vet Intern Med* 2003;17:73-83.

6.   Laste NJ, Harpster NK. A retrospective study of 100 cases of feline distal aortic thromboembolism: 1977-1993. *J Am Anim Hosp Assoc* 1995;31:492-500.

7.   Smith CE, Rozanski EA, Freeman LM, et al. Use of low molecular weight heparin in cats: 57 cases (1999-2003). *Journal Of The American Veterinary Medical Association* 2004;225:1237-1241.

8.   Stokol T, Brooks M, Rush JE, et al. Hypercoagulability in cats with cardiomyopathy. *J Vet Intern Med* 2008;22:546-552.

9.   Kubitza D, Becka M, Voith B, et al. Safety, pharmacodynamics, and pharmacokinetics of single doses of BAY 59-7939, an oral, direct factor Xa inhibitor. *Clin Pharmacol Ther* 2005;78:412-421.

10.   Garcia D, Libby E, Crowther MA. The new oral anticoagulants. *Blood* 2010;115:15-20.

11.   Patel MR, Mahaffey KW, Garg J, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med* 2011;365:883-891.

12.   Landman GW, Gans RO. Oral rivaroxaban for symptomatic venous thromboembolism. *N Engl J Med* 2011;364:1178; author reply 1178.

13.   Moore KT, Plotnikov AN, Thyssen A, et al. Effect of multiple doses of omeprazole on the pharmacokinetics, pharmacodynamics, and safety of a single dose of rivaroxaban. *J Cardiovasc Pharmacol* 2011;58:581-588.

14.   Samama MM, Guinet C, Le Flem L, et al. Measurement of dabigatran and rivaroxaban in primary prevention of venous thromboembolism in 106 patients, who have undergone major orthopedic surgery: an observational study. *J Thromb Thrombolysis* 2013;35:140-146.

15.   Freyburger G, Macouillard G, Labrouche S, et al. Coagulation parameters in patients receiving dabigatran etexilate or rivaroxaban: two observational studies in patients undergoing total hip or total knee replacement. *Thromb Res* 2011;127:457-465.

16.   Eriksson BI, Borris LC, Dahl OE, et al. Dose-escalation study of rivaroxaban (BAY 59-7939)--an oral, direct Factor Xa inhibitor--for the prevention of venous thromboembolism in patients undergoing total hip replacement. *Thromb Res* 2007;120:685-693.

17.	Cuker A, Siegal DM, Crowther MA, et al. Laboratory measurement of the anticoagulant activity of the non-vitamin K oral anticoagulants. *J Am Coll Cardiol* 2014;64:1128-1139.

18.	Miyares MA, Davis K. Newer oral anticoagulants: a review of laboratory monitoring options and reversal agents in the hemorrhagic patient. *Am J Health Syst Pharm* 2012;69:1473-1484.

19.	Samama MM, Contant G, Spiro TE, et al. Laboratory assessment of rivaroxaban: a review. *Thromb J* 2013;11:11.

20.	Alwood AJ, Downend AB, Brooks MB, et al. Anticoagulant effects of low-molecular-weight heparins in healthy cats. *J Vet Intern Med* 2007;21:378-387.

21.	Hirsh J, Bauer KA, Donati MB, et al. Parenteral anticoagulants: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 2008;133:141S-159S.

22.	Walenga JM, Hoppensteadt D, Fareed J. Laboratory monitoring of the clinical effects of low molecular weight heparins. *Thromb Res Suppl* 1991;14:49-62.

23.	Mischke R, Grebe S. The correlation between plasma anti-factor Xa activity and haemostatic tests in healthy dogs, following the administration of a low molecular weight heparin. *Res Vet Sci* 2000;69:241-247.

24.	Mega JL, Braunwald E, Mohanavelu S, et al. Rivaroxaban versus placebo in patients with acute coronary syndromes (ATLAS ACS-TIMI 46): a randomised, double-blind, phase II trial. *The Lancet* 2009;374:29-38.

25.	Ufer M. Comparative efficacy and safety of the novel oral anticoagulants dabigatran, rivaroxaban and apixaban in preclinical and clinical development. *Thromb Haemost* 2010;103:572-585.

26.	Van De Wiele CM, Hogan DF, Green HW, 3rd, et al. Antithrombotic effect of enoxaparin in clinically healthy cats: a venous stasis model. *J Vet Intern Med* 2010;24:185-191.

27.	Arbel Y, Birati EY, Finkelstein A, et al. Platelet inhibitory effect of clopidogrel in patients treated with omeprazole, pantoprazole, and famotidine: a prospective, randomized, crossover study. *Clin Cardiol* 2013;36:342-346.

28.	Kubitza D, Becka M, Roth A, et al. The influence of age and gender on the pharmacokinetics and pharmacodynamics of rivaroxaban--an oral, direct Factor Xa inhibitor. *J Clin Pharmacol* 2013;53:249-255.

29.	Ageno W, Gallus AS, Wittkowsky A, et al. Oral anticoagulant therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012;141:e44S-88S.

30.	Samama MM, Martinoli JL, LeFlem L, et al. Assessment of laboratory assays to measure rivaroxaban--an oral, direct factor Xa inhibitor. *Thromb Haemost* 2010;103:815-825.

31.	Weinz C, Buetehorn U, Daehler HP, et al. Pharmacokinetics of BAY 59-7939--an oral, direct Factor Xa inhibitor--in rats and dogs. *Xenobiotica* 2005;35:891-910.

32.	Biemond BJ, Perzborn E, Friederich PW, et al. Prevention and treatment of experimental thrombosis in rabbits with rivaroxaban (BAY 597939) – an oral, direct factor Xa inhibitor. *Thrombosis and Haemostasis* 2007.

33.   Perzborn E, Strassburger J, Wilmen A, et al. In vitro and in vivo studies of the novel antithrombotic agent BAY 59-7939--an oral, direct Factor Xa inhibitor. *J Thromb Haemost* 2005;3:514-521.

34.   Mueck W, Becka M, Kubitza D, et al. Population model of the pharmacokinetics and pharmacodynamics of rivaroxaban--an oral, direct factor xa inhibitor--in healthy subjects. *Int J Clin Pharmacol Ther* 2007;45:335-344.

35.   Abdulsattar Y, Bhambri R, Nogid A. Rivaroxaban (xarelto) for the prevention of thromboembolic disease: an inside look at the oral direct factor xa inhibitor. *P T* 2009;34:238-244.

36.   Wittkowsky AK. New oral anticoagulants: a practical guide for clinicians. *J Thromb Thrombolysis* 2010;29:182-191.

37.   Kubitza D, Becka M, Wensing G, et al. Safety, pharmacodynamics, and pharmacokinetics of BAY 59-7939--an oral, direct Factor Xa inhibitor--after multiple dosing in healthy male subjects. *Eur J Clin Pharmacol* 2005;61:873-880.

38.   Mueck W, Borris LC, Dahl OE, et al. Population pharmacokinetics and pharmacodynamics of once- and twice-daily rivaroxaban for the prevention of venous thromboembolism in patients undergoing total hip replacement. *Thromb Haemost* 2008;100:453-461.

39.   Eriksson BI, Borris L, Dahl OE, et al. Oral, direct Factor Xa inhibition with BAY 59-7939 for the prevention of venous thromboembolism after total hip replacement. *J Thromb Haemost* 2006;4:121-128.

40.   Turpie AG, Fisher WD, Bauer KA, et al. BAY 59-7939: an oral, direct factor Xa inhibitor for the prevention of venous thromboembolism in patients after total knee replacement. A phase II dose-ranging study. *J Thromb Haemost* 2005;3:2479-2486.

41.   Quinlan DJ, Eriksson BI. Novel oral anticoagulants for thromboprophylaxis after orthopaedic surgery. *Best Pract Res Clin Haematol* 2013;26:171-182.

CHAPTER 6

CONCLUSION

There is a strong medical need for a safe, effective and easily monitored anticoagulant for use in cats that are predisposed to arterial thromboembolism or have previously suffered a thrombotic event. The ideal anticoagulant would not interact with other drugs or foods, and would have predictable pharmacodynamics, be administered orally, and be associated with minimal adverse events. In our *in vitro* study, RVX was shown to have predictable anticoagulant activity in healthy cat plasma. In particular there was a sustained, dose-dependent prolongation of PT and dPT and dose-dependent increase in anti-FXa activity. The dPT assay was more sensitive at detecting lower RVX concentrations than the PT assay. The aPTT test was only mildly prolonged from baseline at differing plasma RVX concentrations and was not sensitive for detection of low concentrations of RVX. Monitoring of anticoagulant effects can be done through measurement of dPT, PT, and anti-Xa levels. However, dPT and anti-Xa levels are the most sensitive detectors of low plasma RVX concentrations. *In vitro* RVX caused dose-dependent hypocoagulability changes in TEG parameters at high RVX; however, kaolin-activated TEG did not appear to be sensitive to low concentrations of RVX in the cat.

Demonstration of the *in vitro* efficacy of RVX led to the second study designed to determine the pharmacodynamics and safety of RVX in two healthy cats after single IV doses. This pilot *in vivo* study of 2 cats demonstrated that IV RVX was viable and

safe. IV dosing caused predictable prolongation of coagulation parameters. The dPT and PT tests appear to be more sensitive to the anticoagulant effects of RVX than aPTT. This is consistent with our previous *in vitro* results of the effect of RVX on coagulation parameters.

The pharmacokinetic and pharmacodynamic profile of RVX after single and multiple oral doses of RVX in healthy adult cats were then evaluated. In addition, adverse effects associated with both short- and long-term administration of RVX were evaluated. Oral RVX was well tolerated in healthy adult cats at a number of different doses and dosing regimens. We found significant correlations between RVX dosage and anti-Xa, and significant differences in mean peak anti-Xa in response to 1.25-mg, 2.5-mg, and 5-mg doses. Clotting times were prolonged from baseline at the time of peak plasma RVX, with a direct correlation between dPT and anti-Xa. All RVX doses resulted in anti-Xa within the target range for human thromboprophylaxis, although at lower doses the target activities were not sustained. Target concentrations for anti-Xa have been developed for the prevention and treatment of various thrombotic syndromes in people. These concentrations have been extrapolated for use in both dogs and cats, although outcome-based recommendations for optimal anti-Xa activity have not been established yet in these species.[182]

Similar to people, peak plasma RVX concentration and anti-Xa activity after oral administration occurred at 3 hours in cats. In the 2.5 mg and 5 mg dose groups, anticoagulant effects had returned to baseline by 24 hours. In this study, anti-Xa was not maintained in the human therapeutic range for the duration of a 12-hour or 24-hour dosing interval. Even with once-daily dosing, which results in a portion of the day with

low anti-Xa activity, human studies have shown positive outcomes in thrombophilic conditions.[183] The pharmacodynamics of RVX in once-daily human trials mirrors the pharmacodynamics seen in healthy cats in the current study. It is not known whether this dosing regimen in cats would prevent primary or secondary thromboembolism or whether a dose retaining FXa activity for 24 hours is more preferable. Alternatively, lower doses of RVX may be indicated to prevent adverse bleeding events if combined with antiplatelet therapy, but this was not specifically evaluated in this study. Future, outcome-based studies will be needed to ascertain this information.

Cats that were administered 1.25 mg RVX orally once daily for 28 days had no residual drug effect seen in the pharmacodynamic profile of the drug. It is possible that co-administration with inhibitors of p-glycoprotein could raise plasma concentrations of RVX and prolong or intensify anticoagulant effects. The possibility also exists that co-administration of RVX with inhibitors of the P450 system could result in increased drug excretion and clearance and alter the therapeutic effect. Future pharmacokinetic and pharmacodynamic studies investigating the concurrent use of RVX with p-glycoprotein inhibitors and P450 inhibitors are warranted. There did not appear to be adverse systemic effects of either the short-term or long-term dosing regimens tested in this study, and no abnormalities were detected on CBC, serum biochemical analysis, and urinalysis testing during this time frame.

We found a statistically significant correlation between RVX dosage, anti-Xa, and dPT, while there was no correlation between dosage and aPTT. Measurements of anti-Xa and dPT may therefore prove useful in future clinical trials to develop RVX dosage regimens for cats. However, the use of dPT requires additional study. The commercially

available kit to measure plasma RVX concentration using a chromogenic assay had excellent correlation with plasma RVX concentrations determined by LC-MS/MS, and is accurate throughout a wide concentration range.[184] While based on measurements of anti-Xa, this assay requires a specific RVX calibrator that increases its cost and limits it use to RVX monitoring. The optimal approach to monitor RVX anticoagulant action, and ultimately develop safe and effective regimens for thromboprophylaxis in cats with HCM will require clinical trials.

In conclusion, oral RVX was safe and well tolerated in healthy cats. Oral administration resulted in predictable plasma drug concentration within the target range for effective thromboprophylaxis in human beings and caused dose-dependent prolongation of dPT. Oral administration of RVX also caused a more reliable dose-dependent increase in anti-Xa concentrations. Our study suggests that RVX holds promise for thromboprophylaxis in cats with heart disease and that clinical trials in these patients are warranted.

REFERENCES

1.   Kol A, Borjesson DL. Application of thrombelastography/thromboelastometry to veterinary medicine. *Vet Clin Pathol* 2010;39:405-416.

2.   Winter RL, Sedacca CD, Adams A, et al. Aortic thrombosis in dogs: presentation, therapy, and outcome in 26 cases. *J Vet Cardiol* 2012;14:333-342.

3.   Song J, Drobatz KJ, Silverstein DC. Retrospective evaluation of shortened prothrombin time or activated partial thromboplastin time for the diagnosis of hypercoagulability in dogs: 25 cases (2006-2011). *J Vet Emerg Crit Care (San Antonio)* 2016;26:398-405.

4.   Abdulsattar Y, Bhambri R, Nogid A. Rivaroxaban (xarelto) for the prevention of thromboembolic disease: an inside look at the oral direct factor xa inhibitor. *P T* 2009;34:238-244.

5.   Atkins CE, Gallo AM, Kurzman ID, et al. Risk factors, clinical signs, and survival in cats with a clinical diagnosis of idiopathic hypertrophic cardiomyopathy: 74 cases (1985-1989). *J Am Vet Med Assoc* 1992;201:613-618.

6.   Rush JE, Freeman LM, Fenollosa NK, et al. Population and survival characteristics of cats with hypertrophic cardiomyopathy: 260 cases (1990-1999). *J Am Vet Med Assoc* 2002;220:202-207.

7.   Tilley LP. Cardiomyopathy and thromboembolism in the cat. *Vet Med Small Anim Clin* 1975;70:313-316.

8.   Tilley LP, Liu SK, Gilbertson SR, et al. Primary myocardial disease in the cat. A model for human cardiomyopathy. *Am J Pathol* 1977;86:493-522.

9.   Baker LD, Birk P. Removal of aortic thrombi in the cat. *Mod Vet Pract* 1974;55:303.

10.   Srinivasan D, Watzak B. Anticoagulant use in real time. *J Pharm Pract* 2013;26:270-279.

11.   Tanaka KA, Key NS, Levy JH. Blood coagulation: hemostasis and thrombin regulation. *Anesth Analg* 2009;108:1433-1446.

12.   Quinlan DJ, Eriksson BI. Novel oral anticoagulants for thromboprophylaxis after orthopaedic surgery. *Best Pract Res Clin Haematol* 2013;26:171-182.

13.   Mega JL, Braunwald E, Mohanavelu S, et al. Rivaroxaban versus placebo in patients with acute coronary syndromes (ATLAS ACS-TIMI 46): a randomised, double-blind, phase II trial. *The Lancet* 2009;374:29-38.

14.   Kubitza D, Becka M, Voith B, et al. Safety, pharmacodynamics, and pharmacokinetics of single doses of BAY 59-7939, an oral, direct factor Xa inhibitor. *Clin Pharmacol Ther* 2005;78:412-421.

15.   Wolf PA, Dawber TR, Thomas HE, Jr., et al. Epidemiologic assessment of chronic atrial fibrillation and risk of stroke: the Framingham study. *Neurology* 1978;28:973-977.

16.   Smith SA. Antithrombotic therapy. *Top Companion Anim Med* 2012;27:88-94.

17.   Couto CG. Disorders of hemostasis In: Nelson RW,Couto CG, eds. *Small animal internal medicine*. St. Louis: Mosby, 2004.

18.   Desai UR. New antithrombin-based anticoagulants. *Med Res Rev* 2004;24:151-181.

19.   Smith SA. Overview of hemostasis In: Weiss DJ, Wardrop KJ, Schalm OW, et al., eds. *Schalm's veterinary hematology*. 6th ed. Ames, Iowa: Wiley-Blackwell,, 2010;1 online resource (xxiii, 1206 p.).

20.   Smith SA. The cell-based model of coagulation. *J Vet Emerg Crit Care (San Antonio)* 2009;19:3-10.

21.   White HD, Gersh B.J, Opie L.H. Antithrombotic agents: platelet inhibitors, anticoagulants and fibrinolytics In: Opie LH,Gersh BJ, eds. *Drugs for the Heart*. 6th ed. Philadelphia: Elsevier Saunders, 2005;xvii, 437 p.

22.   Virchow R. *Thrombosis and emboli (1846-1856)*. Canton, Mass: Science History Publications, 1998.

23.   Dunn M, Brooks, M.B. Antiplatelet and Anticoagulant Therapy In: Bonagura JD, Twedt, D.C., ed. *Kirk's current veterinary therapy XIV*. St. Louis, Mo.: Elsevier Saunders, 2009.

24.   Giorgi MA, Miguel LS. Rivaroxaban in atrial fibrillation. *Vasc Health Risk Manag* 2012;8:525-531.

25.   Wiinberg B, Jensen AL, Johansson PI, et al. Thromboelastographic evaluation of hemostatic function in dogs with disseminated intravascular coagulation. *J Vet Intern Med* 2008;22:357-365.

26.   Kristensen AT, Wiinberg B, Jessen LR, et al. Evaluation of human recombinant tissue factor-activated thromboelastography in 49 dogs with neoplasia. *J Vet Intern Med* 2008;22:140-147.

27.   Sinnott VB, Otto CM. Use of thromboelastography in dogs with immune-mediated hemolytic anemia: 39 cases (2000-2008). *J Vet Emerg Crit Care (San Antonio)* 2009;19:484-488.

28.   de Laforcade A. Diseases associated with thrombosis. *Top Companion Anim Med* 2012;27:59-64.

29.   Nakamura RK, Rishniw M, King MK, et al. Prevalence of echocardiographic evidence of cardiac disease in apparently healthy cats with murmurs. *J Feline Med Surg* 2011;13:266-271.

30.   Paige CF, Abbott JA, Elvinger F, et al. Prevalence of cardiomyopathy in apparently healthy cats. *J Am Vet Med Assoc* 2009;234:1398-1403.

31.   Payne JR, Brodbelt DC, Luis Fuentes V. Cardiomyopathy prevalence in 780 apparently healthy cats in rehoming centres (the CatScan study). *J Vet Cardiol* 2015;17 Suppl 1:S244-257.

32.   Laste NJ, Harpster NK. A retrospective study of 100 cases of feline distal aortic thromboembolism: 1977-1993. *J Am Anim Hosp Assoc* 1995;31:492-500.

33.   Smith SA, Tobias AH, Jacob KA, et al. Arterial thromboembolism in cats: acute crisis in 127 cases (1992-2001) and long-term management with low-dose aspirin in 24 cases. *J Vet Intern Med* 2003;17:73-83.

34.   Hogan DF, Fox PR, Jacob K, et al. Secondary prevention of cardiogenic arterial thromboembolism in the cat: The double-blind, randomized, positive-controlled feline arterial thromboembolism; clopidogrel vs. aspirin trial (FAT CAT). *J Vet Cardiol* 2015;17 Suppl 1:S306-317.

35.   Panetta T, Thompson JE, Talkington CM, et al. Arterial embolectomy: a 34-year experience with 400 cases. *Surg Clin North Am* 1986;66:339-353.

36.   Onder H, Oguzkurt L, Gur S, et al. Endovascular treatment of infrarenal abdominal aortic lesions with or without common iliac artery involvement. *Cardiovasc Intervent Radiol* 2013;36:56-61.

37.   Stokol T, Brooks M, Rush JE, et al. Hypercoagulability in cats with cardiomyopathy. *J Vet Intern Med* 2008;22:546-552.

38.   McMichael MA, Freeman LM, Selhub J, et al. Plasma homocysteine, B vitamins, and amino acid concentrations in cats with cardiomyopathy and arterial thromboembolism. *J Vet Intern Med* 2000;14:507-512.

39.   Casaclang-Verzosa G, Gersh BJ, Tsang TS. Structural and functional remodeling of the left atrium: clinical and therapeutic implications for atrial fibrillation. *J Am Coll Cardiol* 2008;51:1-11.

40.   Watson T, Shantsila E, Lip GY. Mechanisms of thrombogenesis in atrial fibrillation: Virchow's triad revisited. *Lancet* 2009;373:155-166.

41.   Schober KE, Maerz I. Assessment of left atrial appendage flow velocity and its relation to spontaneous echocardiographic contrast in 89 cats with myocardial disease. *J Vet Intern Med* 2006;20:120-130.

42.   Riza Erbay A, Turhan H, Aksoy Y, et al. Activation of coagulation system in dilated cardiomyopathy: comparison of patients with and without left ventricular thrombus. *Coron Artery Dis* 2004;15:265-268.

43.   De Lorenzo F, Saba N, Kakkar VV. Blood coagulation in patients with chronic heart failure: evidence for hypercoagulable state and potential for pharmacological intervention. *Drugs* 2003;63:565-576.

44.   Yusuf S, The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. . *N Engl J Med* 1991;325:293-302.

45.   Ripley TL, Nutescu E. Anticoagulation in patients with heart failure and normal sinus rhythm. *Am J Health Syst Pharm* 2009;66:134-141.

46.   Tarnow I, Falk T, Tidholm A, et al. Hemostatic biomarkers in dogs with chronic congestive heart failure. *J Vet Intern Med* 2007;21:451-457.

47.   Kumagai K, Fukuchi M, Ohta J, et al. Expression of the von Willebrand factor in atrial endocardium is increased in atrial fibrillation depending on the extent of structural remodeling. *Circ J* 2004;68:321-327.

48.   Peverill RE, Harper RW, Gan TE, et al. Increased left atrial thrombin generation in mitral stenosis is not reflected in arterial prothrombin fragment 1+2 levels. *Clin Sci (Lond)* 2000;98:501-506.

49.   Yamamoto K, Ikeda U, Seino Y, et al. Coagulation activity is increased in the left atrium of patients with mitral stenosis. *J Am Coll Cardiol* 1995;25:107-112.

50.   Bedard C, Lanevschi-Pietersma A, Dunn M. Evaluation of coagulation markers in the plasma of healthy cats and cats with asymptomatic hypertrophic cardiomyopathy. *Vet Clin Pathol* 2007;36:167-172.

51.   Helenski CA, Ross JN, Jr. Platelet aggregation in feline cardiomyopathy. *J Vet Intern Med* 1987;1:24-28.

52.   Lopez JA, Kearon, C., Lee, A.Y.Y. Deep vein thrombosis. *Hematology* 2004;1:439-456.

53.   Petersen BJ, Vahouny GV. Cardiac structure and function in vitamin B-12-deprived rats. *J Nutr* 1975;105:1567-1577.

54.   Folsom AR, Nieto FJ, McGovern PG, et al. Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 1998;98:204-210.

55.   Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med* 1993;329:2002-2012.

56.   Kittleson MD, Kienle RD. Thromboembolic disease In: Kittleson MD,Kienle RD, eds. *Small animal cardiovascular medicine*. St. Louis, MO: Mosby, 1998;p.540-547.

57.   Myers JA, Wittenburg LA, Olver CS, et al. Pharmacokinetics and pharmacodynamics of the factor Xa inhibitor apixaban after oral and intravenous administration to cats. *Am J Vet Res* 2015;76:732-738.

58.   Smith SA, Tobias AH. Feline arterial thromboembolism: an update. *Vet Clin North Am Small Anim Pract* 2004;34:1245-1271.

59.   Borgeat K, Wright J, Garrod O, et al. Arterial thromboembolism in 250 cats in general practice: 2004-2012. *J Vet Intern Med* 2014;28:102-108.

60.   Schoeman JP. Feline distal aortic thromboembolism: a review of 44 cases (1990-1998). *J Feline Med Surg* 1999;1:221-231.

61.   Schaub RG, Meyers KM, Sande RD, et al. Inhibition of feline collateral vessel development following experimental thrombolic occlusion. *Circ Res* 1976;39:736-743.

62.   Schaub RG, Meyers KM, Sande RD. Serotonin as a factor in depression of collateral blood flow following experimental arterial thrombosis. *J Lab Clin Med* 1977;90:645-653.

63.   Butler HC. An investigation into the relationship of an aortic embolus to posterior paralysis in the cat. *J Small Anim Pract* 1971;12:141-158.

64.   Prater MR. Acquired coagulopathy I: Avitaminosis K In: Feldman BF, Zinkl JG, Jain NC, et al., eds. *Schalm's veterinary hematology* 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2000;xxxi, 1344 p.

65.   Ansell J, Hirsh J, Hylek E, et al. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 2008;133:160S-198S.

66.   Neff-Davis CA, Davis LE, Gillette EL. Warfarin in the dog: pharmacokinetics as related to clinical response. *J Vet Pharmacol Ther* 1981;4:135-140.

67.   Smith SA, Kraft SL, Lewis DC, et al. Plasma pharmacokinetics of warfarin enantiomers in cats. *J Vet Pharmacol Ther* 2000;23:329-337.

68.   Smith SA, Kraft SL, Lewis DC, et al. Pharmacodynamics of warfarin in cats. *J Vet Pharmacol Ther* 2000;23:339-344.

69.   Baty CJ, Harpster N. K. Warfarin therapy of the cat at risk of thromboembolism In: Bonagura JD, ed. *Kirk's current veterinary therapy XII Small animal practice*. Philadelphia: W.B. Saunders, 1995.

70.   Moore KE, Morris N, Dhupa N, et al. Retrospective study of streptokinase administration in 46 cats with arterial thromboembolism. *Journal of Veterinary Emergency and Critical Care* 2000;10:245-257.

71.   Lunsford KV, Mackin AJ. Thromboembolic therapies in dogs and cats: an evidence-based approach. *Vet Clin North Am Small Anim Pract* 2007;37:579-609.

72.   Pittman JR, Koenig A, Brainard BM. The effect of unfractionated heparin on thrombelastographic analysis in healthy dogs. *J Vet Emerg Crit Care (San Antonio)* 2010;20:216-223.

73.   Ralph AG, Brainard BM. Update on disseminated intravascular coagulation: when to consider it, when to expect it, when to treat it. *Top Companion Anim Med* 2012;27:65-72.

74.   Qi Y, Zhao G, Liu D, et al. Delivery of therapeutic levels of heparin and low-molecular-weight heparin through a pulmonary route. *Proc Natl Acad Sci U S A* 2004;101:9867-9872.

75.   Erickson M, Hiebert LM, Carr AP, et al. Effect of oral administration of unfractionated heparin (UFH) on coagulation parameters in plasma and levels of urine and fecal heparin in dogs. *Can J Vet Res* 2014;78:193-201.

76.   Abildgaard U. Heparin/low molecular weight heparin and tissue factor pathway inhibitor. *Haemostasis* 1993;23 Suppl 1:103-106.

77.   Bentley AM, Mayhew PD, Culp WT, et al. Alterations in the hemostatic profiles of dogs with naturally occurring septic peritonitis. *J Vet Emerg Crit Care (San Antonio)* 2013;23:14-22.

78.   Donahue SM, Brooks M, Otto CM. Examination of hemostatic parameters to detect hypercoagulability in dogs with severe protein-losing nephropathy. *J Vet Emerg Crit Care (San Antonio)* 2011;21:346-355.

79.   Breuhl EL, Moore G, Brooks MB, et al. A prospective study of unfractionated heparin therapy in dogs with primary immune-mediated hemolytic anemia. *J Am Anim Hosp Assoc* 2009;45:125-133.

80.   Brooks MB. Evaluation of a chromogenic assay to measure the factor Xa inhibitory activity of unfractionated heparin in canine plasma. *Veterinary Clinical Pathology* 2004;33:208-214.

81.   Hirsh J, Bauer KA, Donati MB, et al. Parenteral anticoagulants: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 2008;133:141S-159S.

82.   Babski DM, Brainard BM, Ralph AG, et al. Sonoclot(R) evaluation of single- and multiple-dose subcutaneous unfractionated heparin therapy in healthy adult dogs. *J Vet Intern Med* 2012;26:631-638.

83.   Spinler SA, Wittkowsky AK, Nutescu EA, et al. Anticoagulation monitoring part 2: Unfractionated heparin and low-molecular-weight heparin. *Ann Pharmacother* 2005;39:1275-1285.

84.   Brooks M. Coagulopathies and thrombosis In: Ettinger SJ, ed. *Textbook of veterinary internal medicine : diseases of the dog and cat*. 5th ed. Philadelphia: W.B. Saunders Co., 2000.

85.   Couto CG. Disseminated intravascular coagulation in dogs and cats. *Vet Med* 1999;94.

86.   Mischke RH, Schuttert C, Grebe SI. Anticoagulant effects of repeated subcutaneous injections of high doses of unfractionated heparin in healthy dogs. *Am J Vet Res* 2001;62:1887-1891.

87.   Scott KC, Hansen BD, DeFrancesco TC. Coagulation effects of low molecular weight heparin compared with heparin in dogs considered to be at risk for clinically significant venous thrombosis. *J Vet Emerg Crit Care (San Antonio)* 2009;19:74-80.

88.   Alwood AJ, Downend AB, Brooks MB, et al. Anticoagulant effects of low-molecular-weight heparins in healthy cats. *J Vet Intern Med* 2007;21:378-387.

89.   Kolts RL, Maki HS, Kuehner ME, et al. Enhanced streptokinase-induced thrombolysis using heparin in a rabbit model. *J Invest Surg* 1989;2:431-436.

90.   Smith CE, Rozanski EA, Freeman LM, et al. Use of low molecular weight heparin in cats: 57 cases (1999-2003). *Journal Of The American Veterinary Medical Association* 2004;225:1237-1241.

91.   Kleber FX, Witt C, Vogel G, et al. Randomized comparison of enoxaparin with unfractionated heparin for the prevention of venous thromboembolism in medical patients with heart failure or severe respiratory disease. *Am Heart J* 2003;145:614-621.

92.   Goodman SG, Cohen M, Bigonzi F, et al. Randomized trial of low molecular weight heparin (enoxaparin) versus unfractionated heparin for unstable coronary artery disease: one-year results of the ESSENCE Study. Efficacy and Safety of Subcutaneous Enoxaparin in Non-Q Wave Coronary Events. *J Am Coll Cardiol* 2000;36:693-698.

93.   Harenberg J, Weuster B, Pfitzer M, et al. Prophylaxis of embolic events in patients with atrial fibrillation using low molecular weight heparin. *Semin Thromb Hemost* 1993;19 Suppl 1:116-121.

94.   Young E, Cosmi B, Weitz J, et al. Comparison of the non-specific binding of unfractionated heparin and low molecular weight heparin (Enoxaparin) to plasma proteins. *Thromb Haemost* 1993;70:625-630.

95.   Buller HR, Agnelli G, Hull RD, et al. Antithrombotic therapy for venous thromboembolic disease: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004;126:401S-428S.

96.   Van De Wiele CM, Hogan DF, Green HW, 3rd, et al. Antithrombotic effect of enoxaparin in clinically healthy cats: a venous stasis model. *J Vet Intern Med* 2010;24:185-191.

97.   Mischke R, Fehr M, Nolte I. Efficacy of low molecular weight heparin in a canine model of thromboplastin-induced acute disseminated intravascular coagulation. *Res Vet Sci* 2005;79:69-76.

98.   Lunsford KV, Mackin AJ, Langston VC, et al. Pharmacokinetics of subcutaneous low molecular weight heparin (enoxaparin) in dogs. *J Am Anim Hosp Assoc* 2009;45:261-267.

99.   Mischke R, Schmitt J, Wolken S, et al. Pharmacokinetics of the low molecular weight heparin dalteparin in cats. *Vet J* 2012;192:299-303.

100.   Vargo CL, Taylor SM, Carr A, et al. The effect of a low molecular weight heparin on coagulation parameters in healthy cats. *Can J Vet Res* 2009;73:132-136.

101.   Collignon F, Frydman A, Caplain H, et al. Comparison of the pharmacokinetic profiles of three low molecular mass heparins--dalteparin, enoxaparin and nadroparin--administered subcutaneously in healthy volunteers (doses for prevention of thromboembolism). *Thromb Haemost* 1995;73:630-640.

102.   Mischke R, Schonig J, Doderlein E, et al. Enoxaparin: pharmacokinetics and treatment schedule for cats. *Vet J* 2014;200:375-381.

103.   Bauer KA, Eriksson BI, Lassen MR, et al. Fondaparinux compared with enoxaparin for the prevention of venous thromboembolism after elective major knee surgery. *N Engl J Med* 2001;345:1305-1310.

104.   Eriksson BI, Bauer KA, Lassen MR, et al. Fondaparinux compared with enoxaparin for the prevention of venous thromboembolism after hip-fracture surgery. *N Engl J Med* 2001;345:1298-1304.

105.   Fiakpui NN, Hogan DF, Whittem T, et al. Dose determination of fondaparinux in healthy cats. *Am J Vet Res* 2012;73:556-561.

106.   Siller-Matula JM, Schwameis M, Blann A, et al. Thrombin as a multi-functional enzyme. Focus on in vitro and in vivo effects. *Thromb Haemost* 2011;106:1020-1033.

107.   Garcia D, Libby E, Crowther MA. The new oral anticoagulants. *Blood* 2010;115:15-20.

108.   Testa L, Bhindi R, Agostoni P, et al. The direct thrombin inhibitor ximelagatran/melagatran: a systematic review on clinical applications and an evidence based assessment of risk benefit profile. *Expert Opin Drug Saf* 2007;6:397-406.

109.   Connolly SJ, Ezekowitz MD, Yusuf S, et al. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2009;361:1139-1151.

110.   Sakai M, Ohteki H, Narita Y, et al. Argatroban as a potential anticoagulant in cardiopulmonary bypass-studies in a dog model. *Cardiovasc Surg* 1999;7:187-194.

111.   Eriksson BI, Borris L, Dahl OE, et al. Oral, direct Factor Xa inhibition with BAY 59-7939 for the prevention of venous thromboembolism after total hip replacement. *J Thromb Haemost* 2006;4:121-128.

112.   Eriksson BI, Borris LC, Dahl OE, et al. Dose-escalation study of rivaroxaban (BAY 59-7939)--an oral, direct Factor Xa inhibitor--for the prevention of venous thromboembolism in patients undergoing total hip replacement. *Thromb Res* 2007;120:685-693.

113.   Turpie AG, Fisher WD, Bauer KA, et al. BAY 59-7939: an oral, direct factor Xa inhibitor for the prevention of venous thromboembolism in patients after total knee replacement. A phase II dose-ranging study. *J Thromb Haemost* 2005;3:2479-2486.

114.   Wittkowsky AK. New oral anticoagulants: a practical guide for clinicians. *J Thromb Thrombolysis* 2010;29:182-191.

115.   Weinz C, Buetehorn U, Daehler HP, et al. Pharmacokinetics of BAY 59-7939--an oral, direct Factor Xa inhibitor--in rats and dogs. *Xenobiotica* 2005;35:891-910.

116. Weinz C, Schwarz T, Kubitza D, et al. Metabolism and excretion of rivaroxaban, an oral, direct factor Xa inhibitor, in rats, dogs, and humans. *Drug Metab Dispos* 2009;37:1056-1064.

117. Perzborn E, Strassburger J, Wilmen A, et al. In vitro and in vivo studies of the novel antithrombotic agent BAY 59-7939--an oral, direct Factor Xa inhibitor. *J Thromb Haemost* 2005;3:514-521.

118. Eikelboom JW, Weitz JI. A replacement for warfarin: the search continues. *Circulation* 2007;116:131-133.

119. Colman RW. Are hemostasis and thrombosis two sides of the same coin? *J Exp Med* 2006;203:493-495.

120. Biemond BJ, Perzborn E, Friederich PW, et al. Prevention and treatment of experimental thrombosis in rabbits with rivaroxaban (BAY 597939) – an oral, direct factor Xa inhibitor. *Thrombosis and Haemostasis* 2007.

121. Mueck W, Becka M, Kubitza D, et al. Population model of the pharmacokinetics and pharmacodynamics of rivaroxaban--an oral, direct factor xa inhibitor--in healthy subjects. *Int J Clin Pharmacol Ther* 2007;45:335-344.

122. Kubitza D, Becka M, Wensing G, et al. Safety, pharmacodynamics, and pharmacokinetics of BAY 59-7939--an oral, direct Factor Xa inhibitor--after multiple dosing in healthy male subjects. *Eur J Clin Pharmacol* 2005;61:873-880.

123. Mueck W, Borris LC, Dahl OE, et al. Population pharmacokinetics and pharmacodynamics of once- and twice-daily rivaroxaban for the prevention of venous thromboembolism in patients undergoing total hip replacement. *Thromb Haemost* 2008;100:453-461.

124. Majeed A, Schulman S. Bleeding and antidotes in new oral anticoagulants. *Best Pract Res Clin Haematol* 2013;26:191-202.

125. Ghadimi K, Dombrowski KE, Levy JH, et al. Andexanet alfa for the reversal of Factor Xa inhibitor related anticoagulation. *Expert Rev Hematol* 2016;9:115-122.

126. Siegal DM, Curnutte JT, Connolly SJ, et al. Andexanet Alfa for the Reversal of Factor Xa Inhibitor Activity. *N Engl J Med* 2015;373:2413-2424.

127. Patel MR, Mahaffey KW, Garg J, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med* 2011;365:883-891.

128. Usechak PJ, Bright JM, Day TK. Thrombotic complications associated with atrial fibrillation in three dogs. *J Vet Cardiol* 2012;14:453-458.

129. Eriksson BI, Borris LC, Friedman RJ, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after hip arthroplasty. *N Engl J Med* 2008;358:2765-2775.

130. Kakkar AK, Brenner B, Dahl OE, et al. Extended duration rivaroxaban versus short-term enoxaparin for the prevention of venous thromboembolism after total hip arthroplasty: a double-blind, randomised controlled trial. *Lancet* 2008;372:31-39.

131. Lassen MR, Ageno W, Borris LC, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty. *N Engl J Med* 2008;358:2776-2786.

132. Turpie AG, Lassen MR, Davidson BL, et al. Rivaroxaban versus enoxaparin for thromboprophylaxis after total knee arthroplasty (RECORD4): a randomised trial. *Lancet* 2009;373:1673-1680.

133. Investigators E, Bauersachs R, Berkowitz SD, et al. Oral rivaroxaban for symptomatic venous thromboembolism. *N Engl J Med* 2010;363:2499-2510.

134. Romualdi E, Donadini MP, Ageno W. Oral rivaroxaban after symptomatic venous thromboembolism: the continued treatment study (EINSTEIN-extension study). *Expert Rev Cardiovasc Ther* 2011;9:841-844.

135. Kubitza D, Berkowitz SD, Misselwitz F. Evidence-Based Development and Rationale for Once-Daily Rivaroxaban Dosing Regimens Across Multiple Indications. *Clin Appl Thromb Hemost* 2016;22:412-422.

136. Prandoni P, Noventa F, Ghirarduzzi A, et al. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. *Haematologica* 2007;92:199-205.

137. Investigators E-P, Buller HR, Prins MH, et al. Oral rivaroxaban for the treatment of symptomatic pulmonary embolism. *N Engl J Med* 2012;366:1287-1297.

138. Samama MM, Martinoli JL, LeFlem L, et al. Assessment of laboratory assays to measure rivaroxaban--an oral, direct factor Xa inhibitor. *Thromb Haemost* 2010;103:815-825.

139. Miyares MA, Davis K. Newer oral anticoagulants: a review of laboratory monitoring options and reversal agents in the hemorrhagic patient. *Am J Health Syst Pharm* 2012;69:1473-1484.

140. Samama MM, Contant G, Spiro TE, et al. Laboratory assessment of rivaroxaban: a review. *Thromb J* 2013;11:11.

141. Conversy B, Blais MC, Gara-Boivin C, et al. In Vitro Evaluation of the Effect of Rivaroxaban on Coagulation Parameters in Healthy Dogs. *J Vet Intern Med* 2012;26:776.

142. Brainard B, Cathcart, C.J., Dixon, A.C. In vitro effects of rivaroxaban on feline coagulation indices (abstract). *J Vet Intern Med* 2011;25.

143. Conversy B, Blais MC, Dunn M, et al. Rivaroxaban demonstrates in vitro anticoagulant effects in canine plasma. *Vet J* 2013;198:437-443.

144. Wong PC, Pinto DJ, Zhang D. Preclinical discovery of apixaban, a direct and orally bioavailable factor Xa inhibitor. *J Thromb Thrombolysis* 2011;31:478-492.

145. Lassen MR, Raskob GE, Gallus A, et al. Apixaban versus enoxaparin for thromboprophylaxis after knee replacement (ADVANCE-2): a randomised double-blind trial. *Lancet* 2010;375:807-815.

146. Lassen MR, Raskob GE, Gallus A, et al. Apixaban or enoxaparin for thromboprophylaxis after knee replacement. *N Engl J Med* 2009;361:594-604.

147. Buller H, Deitchman D, Prins M, et al. Efficacy and safety of the oral direct factor Xa inhibitor apixaban for symptomatic deep vein thrombosis. The Botticelli DVT dose-ranging study. *J Thromb Haemost* 2008;6:1313-1318.

148. He K, Luettgen JM, Zhang D, et al. Preclinical pharmacokinetics and pharmacodynamics of apixaban, a potent and selective factor Xa inhibitor. *Eur J Drug Metab Pharmacokinet* 2011;36:129-139.

149. Frost C, Wang J, Nepal S, et al. Apixaban, an oral, direct factor Xa inhibitor: single dose safety, pharmacokinetics, pharmacodynamics and food effect in healthy subjects. *Br J Clin Pharmacol* 2013;75:476-487.

150.   Phillips DR, Conley PB, Sinha U, et al. Therapeutic approaches in arterial thrombosis. *J Thromb Haemost* 2005;3:1577-1589.

151.   Fox CS, Coady, S., Sorlie, P. D., et. al. . Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. *Lancet* 1988;2:349-360.

152.   Fox CS, Coady, S., Sorlie, P. D., et. al. Collaborative overview of randomised trials of antiplatelet therapy--I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Antiplatelet Trialists' Collaboration. *BMJ* 1994;308:81-106.

153.   Fox PR. Evidence for or against efficacy of beta-blockers and aspirin for management of feline cardiomyopathies. *Vet Clin North Am Small Anim Pract* 1991;21:1011-1022.

154.   Behrend EN, Grauer GF, Greco DS, et al. Comparison of the effects of diltiazem and aspirin on platelet aggregation in cats. *J Am Anim Hosp Assoc* 1996;32:11-18.

155.   Cathcart CJ, Brainard BM, Reynolds LR, et al. Lack of inhibitory effect of acetylsalicylic acid and meloxicam on whole blood platelet aggregation in cats. *J Vet Emerg Crit Care (San Antonio)* 2012;22:99-106.

156.   Hamel-Jolette A, Dunn M, Bedard C. Plateletworks: a screening assay for clopidogrel therapy monitoring in healthy cats. *Can J Vet Res* 2009;73:73-76.

157.   Quinn MJ, Fitzgerald DJ. Ticlopidine and clopidogrel. *Circulation* 1999;100:1667-1672.

158.   Gent M, Blakely JA, Easton JD, et al. The Canadian American Ticlopidine Study (CATS) in thromboembolic stroke. *Lancet* 1989;1:1215-1220.

159.   Hogan DF, Andrews DA, Talbott KK, et al. Evaluation of antiplatelet effects of ticlopidine in cats. *Am J Vet Res* 2004;65:327-332.

160.   Committee CS. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. *Lancet* 1996;348:1329-1339.

161.   Yusuf S, Zhao F, Mehta SR, et al. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med* 2001;345:494-502.

162.   Hogan DF, Andrews DA, Green HW, et al. Antiplatelet effects and pharmacodynamics of clopidogrel in cats. *J Am Vet Med Assoc* 2004;225:1406-1411.

163.   Willens HJ. Clopidogrel-induced mixed hepatocellular and cholestatic liver injury. *Am J Ther* 2000;7:317-318.

164.   O'Shea J C, Madan M, Cantor WJ, et al. Design and methodology of the ESPRIT trial: evaluating a novel dosing regimen of eptifibatide in percutaneous coronary intervention. *Am Heart J* 2000;140:834-839.

165.   Popma JJ, Satler LF. Early and late clinical outcome following coronary angioplasty performed with platelet glycoprotein IIb/IIIa receptor inhibition: the EPIC Trial results. *J Invasive Cardiol* 1994;6 Suppl A:19A-28A; discussion 45A-50A.

166.   Dowers K, Bright, J. M. . Anti-aggregatory effects of a GPIIB/IIIA antagonist on feline platelet function (abstract). *J Vet Intern Med* 2000:712.

167.   Bright JM, Dowers, K., Hellyer, P. W. Letter to the editors. *J Vet Intern Med* 2002;16:639.

168.   Thompson MF, Scott-Moncrieff, J. C., Hogan, D. F. . Thrombolytic therapy in dogs and cats. *J Vet Emerg Crit Care (San Antonio)* 2001;11:111-121.

169.   Killingsworth CR, Eyster GE, Adams T, et al. Streptokinase treatment of cats with experimentally induced aortic thrombosis. *Am J Vet Res* 1986;47:1351-1359.

170.   Ramsey CC, Riepe, R. D., Macintire, D. K., et al. . Streptokinase: A practical clot buster? *Proceedings 5th International Veterinary Emergency and Critical Care Symposium* 1996:225-228.

171.   Hedlund LJ, Carlson JE, Urness M, et al. Urokinase-mediated thrombolysis: a dose-response relationship in cats. Work in progress. *J Vasc Interv Radiol* 1991;2:349-352.

172.   Matsuu A, Kanda T, Sugiyama A, et al. Mitral stenosis with bacterial myocarditis in a cat. *J Vet Med Sci* 2007;69:1171-1174.

173.   Koyama H, Matsumoto H, Fukushima RU, et al. Local intra-arterial administration of urokinase in the treatment of a feline distal aortic thromboembolism. *J Vet Med Sci* 2010;72:1209-1211.

174.   investigators TG. An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. . *N Engl J Med* 1993;329:673-682.

175.   Albers GW, Bates VE, Clark WM, et al. Intravenous tissue-type plasminogen activator for treatment of acute stroke: the Standard Treatment with Alteplase to Reverse Stroke (STARS) study. *JAMA* 2000;283:1145-1150.

176.   Welch KM, Rozanski EA, Freeman LM, et al. Prospective evaluation of tissue plasminogen activator in 11 cats with arterial thromboembolism. *J Feline Med Surg* 2010;12:122-128.

177.   Donahue SM, Otto, C.M. Thromboelastography: a tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. *Journal of Veterinary Emergency & Critical Care* 2005;15:9-16.

178.   Dias JD, Norem K, Doorneweerd DD, et al. Use of Thromboelastography (TEG) for Detection of New Oral Anticoagulants. *Arch Pathol Lab Med* 2015;139:665-673.

179.   Marschner CB, Bjornvad CR, Kristensen AT, et al. Thromboelastography results on citrated whole blood from clinically healthy cats depend on modes of activation. *Acta Vet Scand* 2010;52:38.

180.   Epstein KL, Brainard BM, Lopes MA, et al. Thrombelastography in 26 healthy horses with and without activation by recombinant human tissue factor. *J Vet Emerg Crit Care (San Antonio)* 2009;19:96-101.

181.   van Beusekom CD, Schipper L, Fink-Gremmels J. Cytochrome P450-mediated hepatic metabolism of new fluorescent substrates in cats and dogs. *J Vet Pharmacol Ther* 2010;33:519-527.

182.   Mischke R, Grebe S. The correlation between plasma anti-factor Xa activity and haemostatic tests in healthy dogs, following the administration of a low molecular weight heparin. *Res Vet Sci* 2000;69:241-247.

183.   Ufer M. Comparative efficacy and safety of the novel oral anticoagulants dabigatran, rivaroxaban and apixaban in preclinical and clinical development. *Thromb Haemost* 2010;103:572-585.

184.   Cuker A, Siegal DM, Crowther MA, et al. Laboratory measurement of the anticoagulant activity of the non-vitamin K oral anticoagulants. *J Am Coll Cardiol* 2014;64:1128-1139.