Clinical Pharmacokinetic Analysis and Modeling of Tranexamic Acid in Dogs

by

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Abstract

Tranexamic Acid (TXA) is an FDA approved drug for hemophilia patients undergoing dental extractions and women experiencing heavy bleeding during menstrual periods. It is also used to stop bleeding and decrease risk of hyperfibrinolysis during surgeries and in patients with injuries. TXA competitively binds to plasminogen at the lysine residue's site preventing its activation to plasmin. This inhibits fibrinolysis and maintains clots for longer times. Hyperfibrinolysis is an abnormal increase in the rate of clot degradation that increases the risk of acute coagulopathy of trauma and shock. This acute condition is induced by a hemostatic imbalance leading to increased blood loss and increased mortality. We determined TXA pharmacokinetics in dogs following single dose administration in 6 healthy mix-breed dogs receiving 10 mg/Kg 10 minutes IV infusion, 20 mg/Kg 10 minutes IV infusion, 15 mg/Kg PO, and 20 mg/Kg PO in a randomized cross-over design using non-compartmental and computational methods to examine different dosing schedules. The overall goal of this research project was to assist investigators in designing further clinical studies to establish the TXA exposure-response relationships and optimize the clinical the effect of different dosing regimens.

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List of Abbreviations

ACOTS	Acute Coagulopathy of Trauma and Shock
ADP	Adenosine diphosphate
A2AP	Alpha 2 Antiplasmin
ACA	Aminocaproic Acid
AFASAK	Copenhagen Atrial Fibrillation, Aspirin and Anticoagulation
AUC	Area under the plasma drug concentration-versus-time curve
AUC_{0-last}	Area under the plasma drug concentration-versus-time curve from time 0 to the last measured concentration
$AUC_{0-\infty}$	Area under the plasma drug concentration-versus-time curve from time 0 to infinity
BART	Blood Conservation Using Antifibrinolytics in a Randomized Trial
CHANCE	Clopidogrel in High-risk Patients with Acute Nondisabling Cerebrovascular Events
CRASH-2	Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage 2
COX-1	Cyclooxygenase-1
CSS	Corrected sum of squared observations
C_{max}	Maximum plasma concentration
Conc.	concentration
CV%	Coefficient of Variation

DVT	Deep Vein Thrombosis
DTI	Direct Thrombin IIa Inhibitors
FDA	Food and Drug Administration
hr	Hour
IC	intracoronary
IV	intravenous
Kg	Kilogram
μg	Microgram
LMWH	Low Molecular Wight Heparin
mg	Milligram
mL	Milliliter
min.	Minute
NCA	Non-compartmental Analysis
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
PO	Oral
aPTT	Activated Partial Prothrombin Time
PT	Prothrombin Time
PCI	Percutaneous Coronary Intervention
PE	Pulmonary Embolism
PK	Pharmacokinetic
PD	Pharmacodynamic
PAI	Plasminogen Activator Inhibitor
STEMI	ST-Elevation Myocardial Infarction

TF	Tissue Factor
TAFI	Thrombin Activated Fibrinolysis Inhibitors
TFPI	Tissue Factor Pathway Inhibitor
t-PA	Plasminogen Tissue Activator
T_{max}	Time to Reach Maximum Concentration
$t_{1/2}$	Half life
TPA	Tissue plasminogen activator
TXA	Tranexamic Acid
UFH	Unfractionated Heparin
u-PA	Urokinase Plasminogen Activator
VWF	Von Willebrand Factor
V_d	Volume of Distribution
WOMAN	World Maternal Antifibrinolytic
LY30	Percentage of clot lysis 30 minutes after MA detection
LY60	Percentage of clot lysis 60 minutes after MA detection
LD	Loading dose
MD	Maintenance dose
Sim.	Simulated
MA	Maximum amplitude
PAI	Plasminogen activator inhibitor
Pred.	Predicted

Chapter 1

Brief Review of Coagulation and Fibrinolysis

1.1. Abstract

This project focuses on the effect of tranexamic acid (TXA) on hyperfibrinolysis associated with acute coagulopathy of trauma and shock (ACOTS). However, in order to understand the mechanism of the drug and the pathophysiology of the ACOTS, this chapter discusses the mechanisms underlying the coagulation cascade and the fibrinolysis in relevance to a variety of different related diseases. A discussion of the different drug classes targeting coagulation factors and platelets used in the management of clotting and thrombosis, in addition to other drugs used in management of bleeding disorders and coagulopathy diseases is included.

1.2. Introduction

Coagulation is the process of clot formation and is mediated by a cascade signaling pathways involving several proteolytic enzymes **(Figure 1.1)**. This process is important to stop bleeding, and its activity is counterbalanced by the physiological fibrinolysis to keep the body in a hemostatic balance. The coagulation cascade is initiated either by an intrinsic or extrinsic pathway. The two pathways have different factors involved in the initiation of the cascade, but both share a common pathway resulting in the formation of the fibrin clot. Intrinsically, factor XIIa reacts with factor XI, which then activates factor X and the process of the cascade continues in order to develop the fibrin clot. The active factor ten (Xa) converts prothrombin to thrombin, and thrombin converts the fibrinogen to its active form fibrin. After that, fibrin strands cross-link to form an insoluble clot by factor XIIIa. Instead of factor XII, the extrinsic pathway starts with factor VIIa after interacting with the tissue factor (TF), continuing the coagulation process and leading to the clot formation. The mechanism of coagulation appears to be mediated by several factors during the signaling process making its regulation more complicated.[1]

Many examples such as the endogenous anticoagulants protein C, antithrombin, and tissue factor pathway inhibitor (TFPI) show some parts of the cascade's complexity. When protein C is activated by thrombin, it inhibits factor V and factor VIII, other factors engaged in the coagulation process. Protein C is also involved in cell death and sepsis and can be down regulated by inflammatory cytokines.[1–3] In addition, antithrombin and TFPI regulate the coagulation by their direct and indirect inhibitory effects on many coagulation factors, including factor X, one of the primary factors in the coagulation cascade. However, TFPI is associated with tissue factors in the extrinsic pathway.[1,4] As it appears, plenty of

factors either directly or indirectly influence the coagulation cascade. Moreover, the fibrinolysis also plays a crucial role to maintain hemostatic balance as mentioned earlier, it acts as a counterbalance against coagulation.

After an insoluble clot is formed, plasminogen interacts with a tissue plasminogen activator (t-PA) or urokinase plasminogen activator (u-PA) which converts plasminogen to its active form, plasmin. When attached to the clot at the lysine residue's site, plasmin increases the activity of t-PA and u-PA leading to an increased activation of plasminogen as a positive feedback mechanism for faster degradation of the clot. However, fibrinolysis is regulated by different protease inhibitors such as plasminogen activator inhibitor 1 (PAI-1), plasminogen activator inhibitor 2 (PAI-2), and alpha 2 antiplasmin (A2AP), to avoid excessive activation of plasminogen. Thrombin mediated factors and thrombin activated fibrinolysis inhibitors (TAFI) also bind to fibrin to decrease the binding affinity of plasmin to fibrin. These inhibitory factors along with the rapid metabolism of plasmin and plasminogen are important in regulating fibrinolysis and maintaining a balanced hemostatic state.[5,6] However, hemostatic trauma caused by injuries or surgeries can disrupt that balance increasing the risk of bleeding.

ACOTS is a bleeding disorder caused by severe injuries and surgeries that are associated with greater risk of bleeding and organ damage. Patients with ACOTS are shown to have a four times greater mortality compared to other trauma patients. It is also associated with prolonged hospitalization and an increased necessity for blood transfusions. Adding to the severity of the injury, other factors also contribute to the development of ACOTS. The volume replacement by blood transfusions or by the shifting of interstitial fluids to the intravenous compartment increases the risk of hyperfibrinolysis,

in addition to the major loss of coagulation factors after the bleeding. Hormonal changes, the immune system, acidosis, hypoxia and hypothermia can also lead to hyperfibrinolysis and the overconsumption of coagulation factors. The pathophysiology of the ACOTS can be explained by the disruption of both the coagulation and the fibrinolysis processes as the over activation of the coagulation process leads to the activation of thrombin, and because of the hypoperfusion state, thrombin will be deactivated by thrombomodulin. Furthermore, the negative feedback mechanism of thrombin can activate the endogenous anticoagulation factor Protein C which blocks the extrinsic coagulation pathway and activates tPA by inhibiting PAI-1 leading to hyperfibrinolysis.[7–9]

Bleeding disorders can also be related to congenital diseases causing a deficiency of clotting factors, or abnormalities in platelet count and function. In most of the cases, platelet-related disorders are associated with superficial bleeding such as excessive nose bleeding, heavy menstruation in women and excessive bleeding after dental extractions. Whereas, disorders associated with a deficiency of coagulation factors are linked to deep tissue bleeding, such as muscles and joint bleeding, or deep-soft tissue hemorrhaging. These symptoms may appear as early as in childhood or later during adulthood especially after a surgery or trauma. Due to the variety of enzymes and the complexity of the coagulation process, several diagnostic and screening tests are necessary to identify the underlying etiology of the disease. Measurement of prothrombin time (PT), activated partial prothrombin time (aPTT), and mixing assays help to identify if a bleeding disorder is related to a specific coagulation factor. Whereas platelet count, Von Willebrand Factor (VWF) and platelet function analyzer tests are important to establish if there is an abnormality in platelets.[10]

Vitamin K deficiency is an example of a coagulopathy disease that affects coagulation factors II, VII, IX and X. It is common in newborn patients and can lead to brain damage and death. It is also associated with Warfarin overdosing and liver diseases. Vitamin K deficiency can be treated with prophylactic Vitamin K supplements.[10,11]

Hemophilia is an inherited coagulopathy disease and can be acquired in some rare cases. It is classified into three types, hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency), and hemophilia C (factor XI deficiency) leading to an inability of clot formation. Antifibrinolytics, such as TXA, along with factors concentrates can be used to avoid bleeding in hemophiliac patients undergoing dental extractions or surgical procedures.[5,10,12–14] Major surgeries and traumatic injuries that induce ACOTS can also be avoided by TXA.[7]

TXA is a synthetic lysine analogue used to prevent bleeding. It blocks plasminogen and plasmin from binding to fibrin at the lysine binding site. In addition to TXA, several antihemorrhagic drugs have been developed to directly or indirectly target fibrinolytic factors. Also, other drugs can affect the coagulation cascade process including the blood thinners which are classified as antiplatelet drugs **(Table 1.1)** and anticoagulants **(Table 1.2)**. Blood thinners are not used for bleeding disorders, instead they are used for thrombotic diseases, but they will be discussed along with anti-hemorrhagic agents in this chapter to provide greater insights into their effect on the coagulation cascade and their use in practice.

1.3. Antiplatelet Drugs

Antiplatelets are blood thinners that indirectly interfere with the coagulation process by inhibiting platelet aggregation. Several classes of antiplatelets have been developed for the management of thrombosis with different mechanisms of action.

1.3.1. Cyclooxygenase-1 Inhibitors

Aspirin (acetyl salicylic acid) is one of the most common antiplatelet drugs and is also classified as a non-steroidal anti-inflammatory drug (NSAID). The antiplatelet effect of aspirin is known to inhibiting the thromboxane A2 by acylating cyclooxygenase 1 (COX1).[15] According to a meta-analysis study, several clinical trials showed that aspirin had a beneficial effect on preventing cardiovascular diseases, such as, myocardial infarction and stroke.[16] However, several trials showed that a daily low dose of aspirin (81mg – 100 mg) failed to reduce cardiovascular diseases in type-2 diabetic patients.[17,18] Another study showed daily aspirin failed to decrease mortality associated with cardiovascular diseases among women taking a low-dose of aspirin daily.[19] The development of resistance is another issue associated with 5% to 45% of patients using aspirin, further complicating its usage. However, there are other COX inhibitors that are used for cardiovascular diseases, such as Triflusal that inhibits the production of thromboxane-B2, can be used as an alternative to aspirin for patients who develop aspirin resistance.[20]

1.3.2. Adenosine Diphosphate Inhibitors

Adenosine diphosphate (ADP) inhibitors are antiplatelet agents that block ADP from binding to its receptor P2Y12 irreversibly in the platelet cell to inhibit platelet aggregation.[21–23] Common ADP inhibitors include the second generation ADP inhibitor clopidogrel (also known as Plavix), which is a prodrug that requires metabolic activation by CYP2C19, a cytochrome P450 (CYP450) enzyme in the liver. This metabolic activation process may reduce the effectiveness of the drug among patients with different CYP2C19 genotypes.[24] Another common ADP inhibitor is the third generation ticagrelor which is more potent and faster than the second generation agents as it directly antagonizes the ADP enzyme without the going through the metabolic activation process.[25] ADP inhibitors can be used in combination with aspirin. The CHANCE trial (Clopidogrel in High-risk patients with Acute Non-disabling Cerebrovascular Events) showed that short-term use of clopidogrel in combination with aspirin reduced the reoccurrence of stroke significantly.[26] In contrast, another clinical trial compared the use of the combination therapy *vs.* aspirin alone and showed that the benefits of the combination therapy were not superior to aspirin alone.[27,28]

1.3.3. Phosphodiesterase Inhibitors

Dipyridamole is a phosphodiesterase inhibitor antiplatelet agent that acts as an antithrombotic and vasodilator. Inhibition of the phosphodiesterase leads to the accumulation of adenosine and other modulators that prevent platelet aggregation.[29] A study that compared dipyridamole with clopidogrel showed that both drug agents decreased the risk of embolism when combined with aspirin in patients with carotid stenosis.[30]

1.3.4. Glycoprotein IIb-IIIa Inhibitors

Glycoprotein IIb-IIIa inhibitors are a class of antiplatelet agents that prevents platelet aggregation by blocking IIb-IIIa receptors which are responsible for attaching fibrinogen to the platelets.[31] Abciximab is an FDA approved glycoprotein IIb-IIIa. It is indicated for patients who undergo percutaneous coronary intervention (PCI) with symptoms of ST-elevated myocardial infarction (STEMI). The drug can be administered intravenously (IV) or intracoronary (IC), and studies showed that there were no significant difference between the IV and IC routes.[32] However, while many studies suggested that Abciximab was associated with risk of bleeding and failed to decrease cardiac events, the drug is still recommended to patients that might have developed resistance to aspirin or clopidogrel.[32,33]

1.4. Anticoagulants

Another type of blood thinners are the anticoagulants. They interfere directly with the coagulation enzymes during the process of the cascade, and they are classified as vitamin K antagonists, factor Xa inhibitors, or thrombin IIa inhibitors.

1.4.1. Vitamin K Antagonists

The most well-known vitamin K antagonist is warfarin. This drug inhibits vitamin K dependent coagulation factors II, VII, IX, and X and proteins C and S.[34] Warfarin is known to decrease the risk of thromboembolic events. The Copenhagen AFASAK trial (Atrial Fibrillation, Aspirin and Anticoagulation trial) compared warfarin with aspirin and placebo. The trial showed that warfarin decreased the risk of thromboembolism in patients

with chronic atrial fibrillation.[35] However, this drug has a number of clinically important limitations due to its narrow therapeutic index and its interaction with a vast number of foods and drugs. Warfarin interferes with many CYP450 isoenzymes, mainly CYP2C9 and CYP3A4 leading to variations in systemic drug concentrations between patients which is why individualized dosing schedules are necessary. Due to high protein binding and narrow therapeutic index, the combination of food and drugs that alter protein binding can lead to clinically meaningful changes in blood coagulation and life-threatening toxicities.[36,37]

1.4.2. Unfractionated Heparin and Low Molecular Weight Heparin

Heparin or unfractionated heparin (UFH) are anticoagulants that inhibit Xa, IXa, and XIa. The mechanism of action of heparin is known to be associated with binding to antithrombin leading to a 1000-fold rate increase of its enzymatic activity. Antithrombin is one of the factors involved in the coagulation cascade that inhibits the conversion of prothrombin to thrombin.[38,39] Studies showed that UFH is associated with a decreased risk of deep vein thrombosis (DVT) and pulmonary embolism (PE) among adult medical-surgical intensive care patients.[40] However, a double-blind randomized phase 4 human clinical trial showed that the rate of mortality was similar to those who received a placebo among patients with severe sepsis.[40,41] In addition, UFH is associated with an increased risk of thrombocytopenia, and low molecular weight heparin (LMWH) is shown to lower the risk of thrombocytopenia.[42,43] Thrombocytopenia is an adverse effect that can occur several days after the use of heparin resulting in a decreased platelet count and is associated with a greater risk of thrombotic complications.[43] This could be avoided by using LMWH

since they have greater inhibition effect on Xa factor without direct involvement of thrombin, which is observed in UFH.[44,45]

1.4.3. Direct Factor Xa Inhibitors

Direct factor Xa inhibitors are oral anticoagulants that directly inhibit Xa without interfering with Antithrombin. The necessity of fast acting drugs with less drug-drug interactions and greater safety margins encouraged the development of such anticoagulants. These oral agents have an advantage over warfarin as they do not require extensive monitoring but, dosing adjustments for patients with renal impairments is necessary. However, they still might interact with drugs that are substrate for CYP 3A4 and P-glycoprotein. The FDA-approved direct oral Xa inhibitor rivaroxaban (Xarelto) has been shown to be better than the non-oral enoxaparin (Lovenox) in the prevention of DVT among patients undergoing hip or knee replacements.[46] Additionally, in a randomized clinical trial comparing the direct Xa inhibitor rivaroxaban with warfarin among patients with mild ischemic stroke and atrial fibrillation, rivaroxaban achieved similar results to warfarin in the prevention of new ischemic lesions. Also, the risk of intracranial hemorrhage did not differ from warfarin. In addition to the previously mentioned advantages of direct Xa inhibitors, the hospitalization period was decreased significantly with rivaroxaban when compared with warfarin, suggesting that rivaroxaban is a suitable anticoagulant to replace the clinical use of warfarin.[47]

1.4.4. Direct Thrombin IIa Inhibitors

Thrombin has three binding sites (the active site, exocite 1, and heparin binding site exocite 2). Thrombin is one of the main factors involved in the coagulation cascade, and mainly responsible for converting fibrinogen to the insoluble fibrin clot. Direct thrombin IIa inhibitors (DTIs) bind to the thrombin's active site and exocite 1, preventing thrombin from activating fibrin, and unlike heparin, it does not require bridging with an antithrombin cofactor, which is why they are called direct thrombin inhibitors. In a meta-analysis comparing the benefit of DTIs with heparin, the analysis showed that DTIs reduced mortality and recurrence of myocardial infarction within 30 days.[48] However, other studies showed that some DTIs had a slightly higher death rate and greater risk of bleeding.[49]

1.5. Antihemorrhagics

Antihemorrhagics are drugs or blood supplements that are used to treat coagulopathy by targeting platelet aggregation or by enhancing coagulation factors. Vitamin K dietary supplements, fresh frozen plasma, coagulation factor concentrates and antifibrinolytics are used as antihemorrhagics along with other drugs. Also, some of the antihemorrhagics used for surgical wounds are available in topical dosage forms.

1.5.1. Factor replacement

Factor replacement can be performed by giving factor concentrates or fresh frozen plasma to replace the missing factors in patients with hemophilia.[10] Also, vitamin K can be used since it is responsible for the production of coagulation factors II, VII, IX and X. Vitamin K is available in a fat-soluble dietary supplement form found in green-leafy

vegetables, or a water-soluble synthetic drug. It is used in patients with vitamin K deficiency especially in neonates.[50,51]

1.5.2. Rituximab

Rituximab is a monoclonal antibody drug that has been effectively and safely used in management of non-Hodgkin B-cells lymphoma and autoimmune diseases such as rheumatoid arthritis and idiopathic membranous nephropathy.[52–54] Growing evidence suggests that Rituximab can be beneficial in the management of non-severe hemophilia A as a first line treatment after a retrospective study showed that Rituximab eliminated inhibitors of Factor VIII.[55] It is known that the drug binds to the CD20 antigen on the surface of B cells, while the cytotoxic mechanism of the drug is not well-understood.[52]

1.5.3. Local Antihemorrhagics

Local antihemorrhagics are absorbable hemostatic agents used with topical thrombin during surgeries to stop wounds from bleeding. Several types are available and approved to be effective such as thrombin spray, gelatin sponge, oxidized regenerated cellulose, microfibrillar collagen and fibrin glue.[56]

1.5.4. Antifibrinolytics

The three known antifibrinolytics are the synthetic lysine analogues aminocaproic acid (ACA) and TXA, and the serine protease inhibitor Aprotinin, which will be discussed below.

1.5.4.1. Aprotinin

This drug is a polypeptide extracted from bovine lungs. It blocks the activation of plasmin and factor XII resulting in the inhibition of both coagulation and fibrinolysis by inhibiting kallikrein; an enzyme responsible for the activation of both plasminogen and factor XII. Aprotinin had shown to decrease risk of bleeding and required blood transfusion in surgical operations.[57] However, the drug has been discontinued worldwide since 2008 after the BART trial (Blood Conservation Using Antifibrinolytics in a Randomized Trial) and several observational studies showed the death rate was greater among patients treated with aprotinin compared to other antifibrinolytics.[58] However, the drug has been approved in some European countries and Canada due to some biases in the BART trial against aprotinin questioning an unexplained exclusion of 137 individuals from the trial and other major limitations.[59]

1.5.4.2. Lysine Analogue Antifibrinolytics

Lysine analogues competitively inhibit plasminogen from binding to the fibrin clot preventing its activation to plasmin. The two known antifibrinolytics are ACA and TXA. Lysine analogues antifibrinolytics are able to stop bleeding even if it is not associated with hyperfibrinolysis, and by their ability to penetrate tissues, they can inhibit tissue fibrinolysis. Although, TXA is ten times more potent than ACA and has a longer half-life, both drugs have very comparable clinical outcomes related to blood loss and blood transfusions with some differences in their efficacy and safety profiles that will be discussed in this section.[57,59]

1.5.4.2.1. Aminocaproic Acid

ACA is a lysine analogue, plasminogen competitive antagonist that inhibits the process of fibrinolysis by binding to plasminogen lysine residuals preventing its activation to plasmin **(Figure 1.2)**. Therefore, limiting the ability of plasminogen and plasmin to degrade fibrin clots. ACA was approved by the Food and Drug Administration in 1964 and is available as an IV dosage form. ACA has a volume of distribution (Vd) of 30 liters with a peak reached after 10 minutes. The drug can penetrate both the extravascular and intravascular compartment enhancing its ability to penetrate red blood cells, tissues and the blood brain barrier. ACA has a terminal half-life of 2 hours and is 65% excreted by the kidneys as an unchanged drug with a clearance of 116 mL/min, but it is decreased in renal failure patients. However, dosing regiments vary based on the type of surgery and severity of bleeding. One of the limitations of ACA is that it has not been evaluated in coagulopathy related to trauma. While, ACA has shown to be effective in reducing blood loss and required blood transfusions in cardiac, liver, and orthopedic surgeries but it must be used with caution in neurosurgeries to avoid the risk of thrombosis. In addition, the chronic use of ACA has shown to increase the risk of myopathy and rhabdomyolysis.[59]

1.5.4.2.2. Tranexamic Acid

TXA is another lysine analogue that also reversibly binds to the lysine residue's site and prevents plasminogen from binding to the fibrin clot **(Figure 1.2)**. This drug is 10 times more potent than ACA. TXA binds to one high affinity site and other weak binding sites in plasminogen. The binding affinity of TXA to the native glutamate terminal

plasminogen (Glu-Plasminogen) is 1.1 μmol/L when it binds to the high affinity site and 750 μmol/L to the weaker sites. For the modified proteolytic the lysine terminal residue (Lys-Plasminogen), the binding affinity to the high affinity site is 2.2 μmol/L, and 36 μmol/L to the weaker sites.[57,60] TXA was effective in decreasing the risk of bleeding, which is responsible for 30% to 40% of death among trauma patients.[59,61] In the CRASH-2 trial, a randomized controlled trial and economic evaluation of the effects of TXA on death, vascular occlusive events and transfusion requirements in bleeding trauma patients showed that the early administration of TXA significantly reduced mortality and risk of death due to bleeding.[62] However, another study showed that the prehospital administration of TXA did not improve overall mortality in trauma patients but the early survival rate and time to death were greater in TXA group than the control.[63] The WOMAN trial (World Maternal Antifibrinolytic) showed that the early use of TXA reduced mortality with no apparent adverse effects among females with postpartum hemorrhage.[64] TXA was approved by the FDA to decrease the risk of hyper-fibrinolysis and bleeding in females with menorrhea and in patients with hemophilia undergoing surgical procedures or dental extraction.[65] While the required concentration to inhibit *in vivo* fibrinolysis is still unknown, a 10 mg/Kg IV bolus dose achieved plasma concentrations of 10 μg/mL and led to 80% *in vitro* inhibition of fibrinolysis. However, complete inhibition of fibrinolysis was obtained only when TXA concentration reached 100 μg/mL. Similar to ACA, dosing guidelines for TXA differs based on the type of surgery or the severity of the bleeding. Regardless of the concentration, a lower dose of TXA (10 mg/Kg bolus followed by 1 mg/kg/hr infusion) was shown to be effective and safe, while larger doses (20-40 mg/Kg bolus followed by 2-4 mg/Kg/hr infusion) failed to show greater beneficial effects.[59] In the BART trial, a TXA

concentrations between 100-150 μg/mL were achieved during operations, and an average concentration of more than 10 μg/mL was seen six hours after operations following a bolus dose of 30 mg/kg (IV bolus) and a maintenance dose of 16 mg/Kg/hr (IV infusion).[66] TXA also showed a decreased risk of blood loss following different surgical operations without thromboembolic complications. TXA saved around 46% of blood in patients undergoing cardiac surgeries and decreased the risk of blood loss in orthopedic surgeries.[59] TXA is 99% excreted from the kidneys as an unchanged drug with a clearance rate of 110-116 ml/min. TXA has an elimination half-life of two hours, and Vd of 9 L. When given orally, it took between 2-3 hours to reach the maximum concentrations, and food appeared to have no influence on the absorption rate with a bioavailability (F) of 34%.[67] Greater risk of seizures have been observed at doses of 100 mg/Kg and greater. Therefore, TXA should be administered with caution in patients with kidney failure. Topical application of TXA has been used in surgical practice and was shown to be as effective as IV administration TXA. Topical use of TXA could be considered in patients with a high risk of seizure but the evaluation of the topical use has not been evaluated for this purpose.[59]

1.6. Objective of the current research

The overall goal of this project was to determine the pharmacokinetics and examine drug exposure-response effects of TXA to better minimize toxicity and improve efficacy. Non-compartmental and computational PK modeling and simulation approaches were used. This work will serve to examine and design further studies to evaluate the effect of different dosing regimens in the future. Regardless of the huge number of studies available in the literature evaluating the use of TXA and other antifibrinolytics in humans, the

motivation of this project was derived from the need of the evaluation of TXA in dogs and animals, as that might reflect the veterinary clinical care to the benefit of dogs with ACOTS and other species of animals.

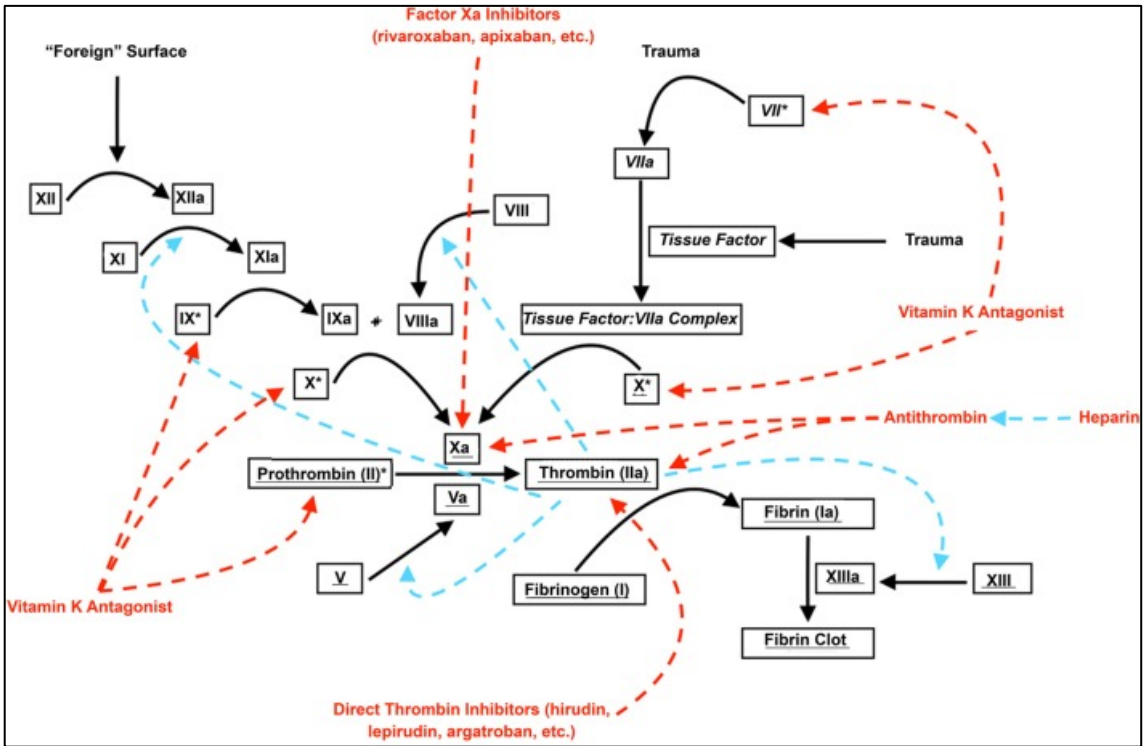
Table 1.1: List of Antiplatelet Drugs

Subclass	**Agents**
Cyclooxygenase-1 Inhibitors	Aspirin, Triflusal
Adenosine Diphosphate Inhibitors[68]	Clopidogrel, Prasugrel, Ticagrelor, Cangrelor, Elinogrel
Phosphodiesterase Inhibitors	Dipyridamole
Glycoprotein IIb-IIIa Inhibitors	Abciximab

Table 1.2: List of Anticoagulants

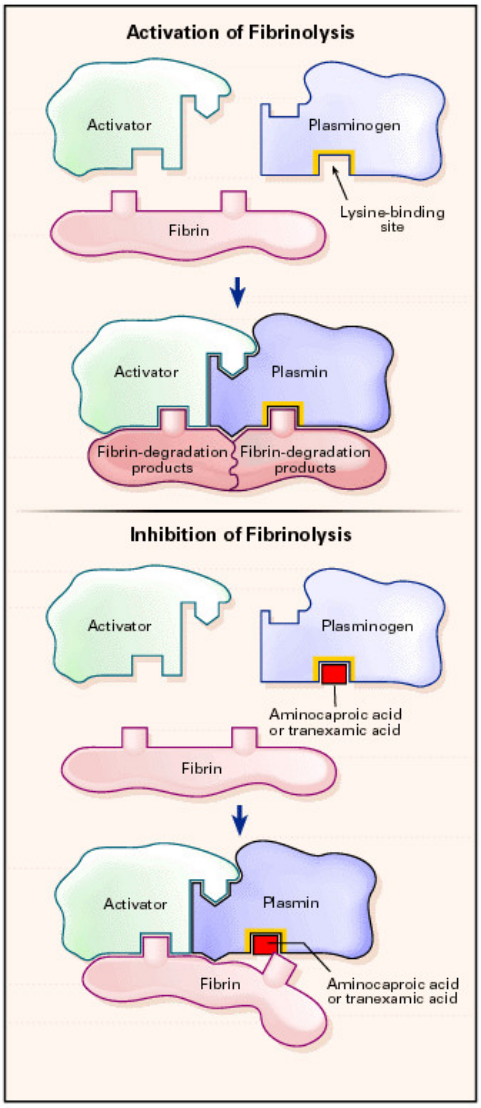
Subclass	**Agents**
Vitamin K Antagonists	Warfarin
Unfractionated Heparin and Low Molecular Wight Heparin[69]	Heparin, Enoxaparin, Fondaparinux, Dalteparin, Tinzaparin
Direct Factor Xa Inhibitors[70]	Rivaroxaban, Apixaban, Edoxaban
Direct Thrombin IIa Inhibitors[71]	Lepirudin, desirudin, Bivalirudin, Argatroban, Dabigartan

Figure 2.1: Coagulation cascade and the effect of anticoagulants. Reprinted with permission from Guan J.[72]



Roman font denotes parts of the "intrinsic" coagulation pathway; italicized font denotes parts of the "extrinsic" coagulation pathway; underlined font denotes parts of the "common" coagulation pathway; red arrow denotes inhibitory effect; blue arrow denotes excitatory effect.

Figure 1.2: Fibrinolysis pathway and TXA mechanism of action. Reprint with permission from Mannucci PM.[57]



In the upper diagram, Plasminogen and t-PA bind to fibrin at the lysine binding site converting plasminogen to plasmin and degrading fibrin to fibrin degradation product. The lower diagram shows TXA binds to plasminogen at the lysine binding site blocking plasminogen from binding and protecting the clot.

Chapter 2

Pharmacokinetics and pharmacodynamics of tranexamic acid in healthy dogs and assessment of antifibrinolytic properties in canine blood in *an in vitro* model of hyperfibrinolysis

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Note

This chapter has been accepted for publication, in part, in the *American Journal of Veterinary Research*. I contributed to this chapter in the pharmacokinetics portion. I applied pharmacokinetics analysis and provided the pharmacokinetics estimated values for all of the four doses received by each dog individually. I have also done calculations to measure the linearity and the bioavailability. Parts of this chapter were modified to fit the structure of the thesis dissertation form and additional information related to the pharmacokinetic analysis have been included that were not included in the submitted paper.

2.1. Abstract

The objective of this study is to assess pharmacodynamics and pharmacokinetics of tranexamic acid (TXA) in dogs and assess antifibrinolytic properties of TXA in canine blood by use of a thromboelastography-based *in vitro* model of hyperfibrinolysis. Six healthy adult dogs received each of 4 TXA treatments (10 mg/kg, IV; 20 mg/kg, IV; 15 mg/kg, PO; and 20 mg/kg, PO) in a randomized crossover-design study. Blood samples were collected at baseline (time 0; immediately prior to drug administration) and predetermined time points afterward for pharmacokinetic analysis and pharmacodynamic (thromboelastography) analysis by use of an *in vitro* hyperfibrinolysis model. Maximum amplitude (MA; representing maximum clot strength) significantly increased from baseline at all time points for all treatments but was lower at 360 minutes for the 10 mg/kg IV treatment than for other treatments. Percentage of clot lysis 30 minutes after MA was detected was significantly decreased from baseline at all time points for all doses; at 360 minutes, this value was higher for the 10 mg/kg IV treatment than for other treatments and higher for the 20 mg/kg IV treatment than for the 20 mg/kg PO treatment. Maximum plasma TXA concentrations were dose dependent. At 20 mg/kg, IV, plasma TXA concentrations briefly exceeded concentrations suggested for complete inhibition of fibrinolysis. Oral drug administration resulted in later peak antifibrinolytic effect than did IV administration. In conclusion, the administration of TXA improved clot strength and decreased fibrinolysis in blood samples from healthy dogs in an *in vitro* hyperfibrinolysis model. Further research is needed to determine the clinical effects of TXA in dogs with hyperfibrinolysis.

2.2. Introduction

Hyperfibrinolysis, or accelerated blood clot breakdown, is associated with a number of disease states, and can lead to morbidity and death because of uncontrolled or recurrent hemorrhage.[62] Hyperfibrinolysis can develop after trauma, contributing to ACOTS. In one study,[9] ACOTS was identified in 266 of 1088 (24.4%) human trauma patients at the time of hospital admission and was associated with a 5-fold increase in mortality rates. Hypoperfusion, shock, and inflammation result in a hypocoagulable and hyperfibrinolytic state in the immediate posttrauma period.[73] Although ACOTS mimics disseminated intravascular coagulation, it is not a consumptive coagulopathy, and the 2 conditions are considered separate entities.[74] Hyperfibrinolysis in ACOTS is partly attributable to an increase in local release of TPA by damaged endothelial cells. In addition, increased circulating concentrations of activated protein C inhibit plasminogen activator inhibitor-1, an inhibitor of the fibrinolysis pathway.[75] Hyperfibrinolysis has also been identified in patients following cardiopulmonary bypass and can result in increased postoperative transfusion requirements.[76] In dogs, hyperfibrinolysis has been reported following severe trauma[77] and development of severe, acute hemoperitoneum.[78] In addition, a hyperfibrinolytic condition is suspected to cause postoperative bleeding in retired racing greyhounds.[79]

Antifibrinolytic agents such as ACA and TXA can slow fibrinolysis and reduce blood loss in patients with hyperfibrinolytic conditions. Both of these drugs are synthetic lysine analogs that reversibly bind to plasminogen, preventing plasmin binding to fibrin.[80] Results of a large, prospective study[1] of human patients with traumatic injuries revealed a significantly lower mortality rate for those treated with TXA, compared with no

antifibrinolytic treatment. Notably, the use of an antifibrinolytic in that study[62] did not result in an increase in the incidence of thrombotic events. Administration of TXA to human patients after cardiac surgery that includes cardiopulmonary bypass reduces hemorrhage by one-third, and this treatment also reduces postoperative bleeding after elective orthopedic procedures.[81] To the authors' knowledge, the use of antifibrinolytic agents has not been evaluated in dogs with hemoperitoneum or ACOTS, but these treatments may be of benefit. Perioperative administration of ACA has been shown to decrease the incidence of postoperative bleeding in retired racing greyhounds undergoing elective limb amputations or gonadectomy.[82,83]

A variety of TXA doses ranging from 10 mg/kg[84] to 50 mg/kg[85] IV have been evaluated in healthy dogs. Such investigations have attempted to evaluate the antifibrinolytic properties of TXA by use of unmodified viscoelastic coagulation testing (thromboelastography or rotational thromboelastometry), with varied results. Although useful for assessing coagulation, these tests show relatively minimal fibrinolysis in blood samples from healthy dogs, making it difficult to accurately assess the pharmacodynamic effects of antifibrinolytic agents. A recently described modified thromboelastography assay that induces an *in vitro* hyperfibrinolytic state[86] has been used to assess the pharmacodynamics of ACA in blood from healthy dogs.[87] Tranexamic acid is 10 times as potent as ACA,[86] and recent backordering of ACA in the United States made TXA the only available antifibrinolytic agent for a period of time. Limited dosing information and unfamiliarity with TXA in this context could potentially compromise veterinary patient care.

The objective of the study reported here was to determine the pharmacokinetic and pharmacodynamic profile of TXA in dogs following administration of a single dose by IV infusion (10 or 20 mg/kg over 10 minutes) or by mouth (15 or 20 mg/kg). We hypothesized that administration of TXA to healthy dogs would result in dose-dependent increases in plasma drug concentrations and antifibrinolytic effects as assessed by use of a modified thromboelastography[87] assay. We also hypothesized that orally administered TXA would have pharmacodynamic characteristics similar to those following IV administration healthy dogs.

2.3. Materials and Methods

2.3.1. Dogs

Six healthy, sexually intact, mixed-breed female dogs between 2 and 3 years of age with a median weight of 14.2 kg (range, 13.5 to 16.3 kg) were included in the study. The dogs were deemed healthy on the basis of results of a physical examination, CBC, serum biochemical analysis, and coagulation analysis, which included prothrombin time, activated partial thromboplastin time, and analysis by TF-activated thromboelastography. The University of Georgia Institutional Animal Care and Use Committee approved all study procedures.

2.3.2. Drug administration and sample collection procedures

Each dog was assigned to receive a single dose of TXA IV (Akorn, Lake Forest, Ill) (10 mg/kg or 20 mg/kg) or PO (Lysteda, Ferring Pharmaceuticals, Parsippany, NJ) (approx 15 or 20 mg/kg) in a randomized, crossover design. Randomization was performed using an

internet-based randomizer program. Each dog received all treatments during the course of the study with a 7-day washout period between treatments. Food was withheld ≥ 12 hours prior to TXA administration.

The IV TXA treatments were given as an infusion over 10 minutes in an effort to minimize the incidence of vomiting.[85] One tablet size (650 mg of TXA in a 1 g tablet) was available for use; the desired dose was calculated as a percentage of the tablet, and tablets were weighed and then divided accordingly. The resulting pieces were weighed to confirm that they would deliver the desired dose, assuming equal distribution of the active ingredient throughout the tablet.

The day prior to drug administration, dogs were sedated with dexmedetomidine (Domitor, Pfizer, New York, NY) (5 µg/kg, IV), and a 20-gauge, 12-cm indwelling catheter (Arrow Teleflex, Telford, Pa) was placed in a jugular vein with a modified Seldinger technique. Following placement, catheters were initially flushed with sterile saline (0.9% NaCl) solution (Hospira, Lake Forest, Ill), and subsequently flushed with 0.5 mL of a 50% dextrose solution[e] to maintain catheter patency and preclude the use of anticoagulant flush. The following day, catheters were flushed with sterile saline solution, and 3 mL of blood was withdrawn and discarded before collection of any blood for analysis. The total volume of blood collected on each sampling day was ≤ 17 mL. Following sample collection, the catheter was flushed with sterile saline solution. Jugular catheters were removed after final sample acquisition. The dogs were observed for clinical signs of adverse reactions during and after administration of the drug, and any clinical effects were recorded.

2.3.3. Pharmacodynamics

Blood samples for pharmacodynamic analysis were collected at time 0 (ie, baseline [immediately prior to drug administration]) and at 60, 240, and 360 minutes after drug administration. Blood was transferred into tubes containing 3.2% sodium citrate solution (BD, Franklin Lakes, NJ) to a final blood-to-citrate ratio of 9:1. Thromboelastography was performed after a standard sample rest time of 30 minutes at room temperature (22° to 24°C) by 1 researcher (BMB), who was blinded to the administered drug dose and route. At each timepoint, a TF-activated thromboelastography assay (TEG 5000, Haemonetics Corp, Braintree, Mass) (TF-thromboelastography; final dilution of TF [Dade Innovin in 2% Albumin, Siemiens Healthcare Diagnostics, Newark, Del], 1:3,400) and a modified hyperfibrinolytic thromboelastography (TPA-thromboelastography, with a final TF dilution of 1:3,400 and a final TPA [Genetech, South San Francisco, Calif] concentration of 100 U/mL) were performed as described elsewhere.[86] All assays were continued for 60 minutes after the MA of the clot was detected. Procoagulant variables (reaction time [R], α-angle, and MA) and fibrinolysis variables were calculated with proprietary software (TEG Software, Version 4.2, Haemonetics Corp, Braintree, Mass), visually inspected for accuracy, and recorded. The fibrinolysis variables were represented as LY30 and LY60, calculated as a ratio of the AUC at MA to the AUC at the specified time point. Additional thromboelastography variables measured and calculated by the software included clot formation time (K) and shear modulus strength (G; calculated as [5,000 X MA]/[100 – MA]).[88]

2.3.4. Pharmacokinetics

Blood samples for pharmacokinetic analysis (1 mL) were collected into EDTA-containing blood tubes[f] at baseline and at 5, 10, 15, 30, 60, 120, 240, and 360 minutes after drug administration. At time points when 2 samples were required, samples were collected sequentially into separate syringes, with the sample for thromboelastography analysis collected first. The EDTA-containing samples were held on ice until centrifugation (1,500 X *g* for 10 minutes at room temperature), which was performed ≤ 1 hour after collection. Plasma supernatant was removed and stored at –80°C for ≤ 4 months until analysis. Samples were batch analyzed by high-performance liquid chromatography–mass spectrometry at a commercial toxicology laboratory (University of Iowa Pharmacology Analytical Support Team, Ames, Ia).

The pharmacokinetics parameters were estimated by noncompartmental analysis from the plasma drug concentration–time profile data following a 10-minute IV infusion or oral administration, by an investigator using commercial software (WinNonlin Professional, Version 5.2, formerly Pharsight Corporation, now Certara USA, St Louis, Mo). Parameters calculated from IV data included C_{max}, t_{max}, $AUC_{0–last}$ (ie, the AUC from time 0 [drug administration] to 6 hours after administration), $AUC_{0–\infty}$, $t_{1/2}$, Vd, and systemic clearance. Parameters following oral drug administration included C_{max}, t_{max}, $AUC_{0–last}$, and $AUC_{0–\infty}$. The C_{max} and t_{max} were determined by examination of the drug concentration–time profiles, and AUC values were estimated by the log-trapezoidal method and the method for extrapolating exposure from the last measurement concentration to infinity. A preliminary assessment of oral bioavailability was performed by comparing the oral $AUC_{0–\infty}$ /oral dose divided by IV $AUC_{0–\infty}$ /IV dose.

2.3.5. Statistical analysis

Statistical analysis was performed with commercial statistical analysis software (Sigma Stat, Systat Inc, San Jose, Calif). Data were found to be normally distributed by use of the Shapiro-Wilk test and are reported as mean ± SD. The influence of TXA dosage or time (baseline, 60 minutes, 240 minutes or 360 minutes) in relation to dosage on thromboelastography variables was evaluated with 2-way repeated-measures ANOVA. Values of $P < 0.05$ were considered significant.

2.4. Results

2.4.1. Adverse events

Five dogs vomited once during administration of the 20 mg/kg IV dose of TXA. Vomiting occurred between 6 and 10 minutes after the start of the infusion. The dog that did not vomit had signs of nausea (lip licking) 8 minutes after the infusion started. For each dog that vomited, no further signs of nausea were noted after emesis. No signs of nausea or vomiting were observed for the same dogs when the 10 mg/kg IV dose or either oral dose was given. No other adverse effects were noted.

2.4.2. Pharmacodynamics

The TF-thromboelastography results for all times and dosages were within the institutional reference ranges (data not shown). There were no significant differences in results among any TF-thromboelastography samples. When TPA-thromboelastography was performed, there was a significant ($P < 0.05$) increase from the time 0 (baseline [immediately prior to drug administration]) values for α-angle and MA at 60, 240, and 360

minutes after TXA administration, regardless of dose or route **(Table 2.1)**. At 360 minutes, the MAs for samples collected after administration of TXA at 15 and 20 mg/kg PO and 20 mg/kg IV were significantly greater than that for samples collected after administration of the drug at 10 mg/kg IV.

The LY30 was significantly ($P < 0.05$) decreased from baseline at all time points after TXA administration for all dosages **(Table 2.2)**. After administration at 10 mg/kg IV, LY30 values at 240 and 360 minutes were greater than that measured at 60 minutes, but were not significantly different from each other. At the 240 minute time point, LY30 for the 10 mg/kg IV treatment was significantly greater than those for the 15 and 20 mg/kg PO treatments. At the 360 minute time point, LY30 was significantly greater for the 10 mg/kg IV treatment than those for all other treatments; LY30 for the 20 mg/kg IV treatment was also significantly greater than that for the 20 mg/kg PO treatment at this time point. For the 15 mg/kg PO treatment, LY30 at 60 minutes was significantly greater than that at 240 minutes.

The LY60 was significantly decreased from the baseline value at all time points after TXA administration at 15 and 20 mg/kg PO and 20 mg/kg IV, but was only decreased at the 60 minute time point relative to baseline after administration at 10 mg/kg IV (Table 2). For the 20 mg/kg PO treatment, LY60 at 60 minutes was significantly greater than that at 240 and 360 minutes. At 240 minutes, LY60 was significantly greater for the 10 mg/kg IV treatment than for the 20 mg/kg IV and 20 mg/kg PO treatments. At 360 minutes, LY60 for the 10 mg/kg IV treatment was significantly greater than those for the 15 and 20 mg/kg PO treatments, and LY60 for the 20 mg/kg IV treatment was significantly greater than that for 20 mg/kg PO treatment.

2.4.3. Pharmacokinetics

Pharmacokinetic parameters were determined after administration of each TXA treatment **(Table 2.3)**. The plasma drug concentration–time profiles were characteristic for short-term IV (10 minutes infusion) and oral drug administration **(Figure 2.1).** An exponential decrease in drug concentration over time was observed following IV administration. The estimated PK parameters, Cl/kg, V/kg, and AUC/dose were similar and suggested that at the IV doses tested TXA kinetics were linear.

Following oral administration, t_{max} occurred at approximately 2 hours for both doses (Figure 1). The C_{max}, $AUC_{0–last}$, and $AUC_{0–\infty}$ were each dose dependent, suggesting that at 15 and 20 mg/kg PO, the pharmacokinetics of TXA were linear. Owing to the lack of sample collection after 6 hours, the extrapolated area under the TXA drug plasma concentration curve following oral drug administration were described as the predicted mean ± SD $AUC_{0\text{-last and}}$ $AUC_{0–\infty}$. Absolute bioavailabilities for the oral doses were not determined because the predicted exposure (i.e., $AUC_{last–\infty}$) following oral dosing represented 41.0 ± 13.7% and 30.7 ± 13.7%% of the total $AUC_{0–\infty}$ for the 15 and 20 mg/kg doses, respectively. However, a preliminary estimation of the bioavailability after oral administration yielded values ranging from 92% to 117%, based on these data.

2.5. Discussion

In the study reported here, use of an *in vitro* model of hyperfibrinolysis revealed that IV and orally administered TXA reduced fibrinolysis in blood samples from healthy dogs. Significant ($P < 0.05$) reductions in LY30, compared with the baseline (time 0; immediately prior to drug administration) values, were found for all tested dosages at 60

through 360 minutes after drug administration. The MAs, or maximum clot strengths, at the same time points were also greater than the respective baseline values for all treatments. These data indicate that treatment with TXA can enhance clot strength and duration in patients with hyperfibrinolysis. The doses administered in this study were based on previously reported veterinary use[84,85,89] and extrapolated from data for human patients.[80]

Although administration of TXA at 10 mg/kg IV resulted in improved maximum clot strength and reduction of clot lysis relative to baseline as measured by both LY30 and LY60 1 hour after injection, the duration of the effect did not last as long as that for other dosages. By 6 hours after administration of this treatment, both MA and LY30 were significantly different from those for other dosages and were returning toward the baseline value. Within this treatment, changes in LY30 indicated a reduced antifibrinolytic effect over time. These data indicated that administration of TXA at 10 mg/kg IV might be needed more frequently than the other doses and routes tested to maintain a similar antifibrinolytic effect.

Oral administration of TXA resulted in delayed, but prolonged, antifibrinolytic effects compared with IV administration. A significant decrement in drug effect was not detected during the observation time after administration of the 20 mg/kg oral dose, and this supported a dosing interval of 6 hours or longer to maintain a clinical effect. In humans, TXA is administered orally every 8 hours, and although samples were not collected 8 hours after drug administration in the present study, it seems that this strategy might be appropriate for dogs as well. An alternative strategy could be to administer a loading dose IV, followed by oral drug administration to maintain prolonged antifibrinolytic effects.

However, further evaluation is needed to determine the duration of the antifibrinolytic effects of TXA administered orally at intervals of 8 hours or longer.

Results of a previous study[86] that involved a similar *in vitro* hyperfibrinolysis model indicated that a TXA concentration of 144.7 μg/mL completely inhibited fibrinolysis in canine plasma. Although dosing recommendations were not made from the results of that study,[86] the 20 mg/kg IV dosage did result in plasma TXA concentrations > 150 μg/mL shortly after administration in the present study. These plasma concentrations were not maintained, although antifibrinolytic effects persisted for several hours after the t_{max}. None of the other dosages investigated in this study approached the target plasma concentration derived from the earlier study, but each achieved a reduction in clot lysis from the baseline value.

The C_{max} of TXA in the present study was dose dependent. The C_{max} for both IV doses was achieved shortly after administration and decreased steadily. Despite decreasing plasma concentrations of TXA, antifibrinolytic effects were still detected up to 6 hours after administration. The C_{max} after oral administration of 15 and 20 mg of TXA/kg was identified at 144 and 120 minutes, respectively; however, more accurate estimates of t_{max} values might have been identified if sampling had been performed at more frequent intervals. The antifibrinolytic effects were more evident at 240 and 360 minutes after oral drug administration, compared to the IV dosages, which had demonstrable antifibrinolytic effects at the 60 minute assay timepoint. Assays performed 8 hours or longer after TXA administration might have been helpful in determining the total duration of effect and evaluating drug clearance. In human patients, TXA is eliminated by glomerular filtration.[90] To the authors' knowledge, no studies have explicitly evaluated excretion of TXA by dogs,

but it is reasonable to postulate a similar elimination profile. Drug clearance and clinical effects of renally excreted medications can be altered in animals with renal dysfunction.

Vomiting, a known adverse effect of TXA, was observed in 5 of 6 dogs during IV infusion of the 20 mg/kg dose in the present study. The dog that did not vomit during this treatment showed signs of nausea. No vomiting or signs of nausea were noted during or after administration of the other dosages. Although each dog only vomited once, any incidence of vomiting, especially in compromised patients, may lead to complications such as development of aspiration pneumonia. Tranexamic acid is thought to induce emesis through activation of the tachykinin neurokinin 1 receptor.[91] Maropitant is an antagonist of tachykinin neurokinin 1 and is routinely used for treatment of nausea and vomiting. It may be useful to prevent vomiting associated with TXA administration; however, this has not been evaluated in veterinary patients. Each IV dose of TXA in our study was administered as a short-term infusion in an attempt to reduce the incidence of vomiting, as previously reported adverse effects were associated with rapid IV bolus administration.[85] Infusion of the drug over a longer period of time would likely alter the C_{max} of the drug, but this would not be expected to reduce the antifibrinolytic properties of the drug, as antifibrinolytic effects were still present at lower plasma drug concentrations. In adult human patients with trauma, TXA is administered as an initial 1-g IV loading dose followed by 1 g given by continuous rate infusion over 8 hours.[62] This type of administration (an approx 14 mg/kg loading dose, followed by 14 mg/kg given over 8 hours, assuming a typical human patient weight of 70 kg) was not evaluated in the present study; however, a similar approach should be evaluated to assess whether it would maintain effective plasma concentrations over a longer period of time and reduce the incidence of vomiting and signs of nausea in dogs.

The present study had several limitations. The *in vitro* model used does not take into account the role of the endothelium in fibrinolysis, and endogenous TPA concentrations in the circulation may be higher or lower than the concentration used in the study. In addition, endothelial-derived inhibitors of fibrinolysis such as plasminogen activator inhibitor 1 are not accounted for in this model. The tablets used for oral TXA administration were not designed to be divided (ie, tablets were not scored); therefore, equal distribution of the drug throughout the tablet could have varied and the administered dose might have been overestimated or underestimated. The mean blood sample collected from each dog each week of testing was 1.12 mL/kg, and thromboelastography parameters can be affected by Hct and platelet concentrations[92]; however, CBCs performed after the third week of testing did not reveal any changes that would be expected to alter these parameters. Only 6 dogs were used in the study, and this small number may have limited the statistical power to detect alterations in all of the thromboelastography parameters evaluated. Based on the observed PK profile, additional samples beyond six hour time point following oral administration would have allowed a more accurate estimate of bioavailability. Furthermore, all of the study dogs were healthy, and thus the pharmacokinetic and pharmacodynamic data may not apply to patients with decreased perfusion or altered Vd. The same dosages of TXA may not have the same degree of inhibitory effect in such patients, particularly in the context of oral drug administration and decreased gastric perfusion. However, these preliminary data suggested that evaluation of the effects of TXA in canine patients with hyperfibrinolytic conditions is warranted.

2.6. Acknowledgements

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Table 2.1: Mean ± SD thromboelastography values for blood samples collected from 6 healthy dogs immediately before (0 minutes) and 60, 240, and 360 minutes after IV or oral administration of TXA and evaluated by use of an *in vitro* model of TPA-induced hyperfibrinolysis in a randomized, crossover-design study with a 1-week washout period between experiments.

Variable	**Reference range**	**Time (min)**	**Oral administration**		**IV administration**	
			15 mg/kg	**20 mg/kg**	**10 mg/kg**	**20 mg/kg**
R (min)	1.8–2.0	0	1.88 ± 0.26	1.733 ± 0.23	1.85 ± 0.33	1.82 ± 0.23
	—	60	1.85 ± 0.22	1.62 ± 0.26	1.77 ± 0.24	1.65 ± 0.15
	—	240	1.68 ± 0.17	1.65 ± 1.22	1.72 ± 0.24	1.65 ± 0.23
	—	360	1.817 ± 0.19	1.72 ± 0.12	1.883 ± 0.53	1.80 ± 0.11
K (min)	1.2–2.0	0	1.267 ± 0.15	2.27 ± 1.40	1.65 ± .021	1.90 ± 1.42
	—	60	0.97 ± 0.14	1.10 ± 0.28	1.183 ± 0.33	1.08 ± 0.33
	—	240	1.05 ± 0.27	1.15 ± 0.29	1.2 ± 0.24	1.13 ± 0.28
	—	360	1.17 ± 0.47	1.22 ± 0.26	2.616 ±2.06	1.12 ± 0.25
α angle (°)	61.1–68.3	0	68.13 ± 4.35	67.68 ± 5.56	62.88 ± 7.99	67.5 ± 8.55
	—	60	75.62 ± 1.45*	74.38 ± 3.78*	72.78 ± 4.10*	74.43 ± 3.75*
	—	240	74.25 ± 3.56*	73.45 ± 3.79*	73.2 ± 2.76*	74.05 ± 3.55*
	—	360	73.13 ± 5.77*	73.00 ± 3.10*	67.45 ± 9.78*	74.12 ± 2.81*
MA (mm)	20.9–30.3	0	21.62 ± 11.29[a]	37.68 ± 14.28[b]	19.6 ± 10.32[a]	25.28 ± 12.64[a]
	—	60	51.85 ± 7.83*	52.67 ± 6.53*	50.7 ± 4.32*	50.15 ± 3.78*
	—	240	55.98 ± 7.74*	54.40 ± 6.63*	52.25 ± 5.91*	53.37 ± 4.96*
	—	360	54.37 ± 7.70*[a]	54.63 ± 6.17*[a]	42.32 ±16.57*†[b]	56.5 ± 4.85*[a]
G (dynes/s)	1,517–2,627	0	1,505 ± 1,044	3,461 ± 2,358	1,307 ± 827	1,847 ± 1,105
	—	60	5,597 ± 1,569*	5,638 ± 1,443*	5,206 ± 880*	5,080 ± 760*
	—	240	6,670 ± 2,141.*	6,161 ± 1,633*	5,594 ± 1,145*	5,819 ± 1,141*

	—	360	6,190 ± 1,713*[a]	6,182 ± 1,427*[a]	4,265 ± 2,544*[b]	6,610 ± 1,177*‡[a]

Values of $P < 0.05$ were considered significant. *Within a column, value differs significantly from the value at 0 minutes. †Within a column, (nonbaseline) value differs significantly from value at 60 and 240 minutes. ‡Within a column, (nonbaseline) value differs significantly from value at 60 minutes. [a,b]Within a row, values with different superscript letters are significantly different.

G = Shear modulus strength. K = Clot kinetic time (defined as the time to reach an amplitude of 20 mm). R = Reaction time. — = Not applicable.

Table 2.2: Mean ± SD clot lysis data for the same samples as in **Table 2.1** as assessed by use of an *in vitro* model of TPA-induced hyperfibrinolysis.

Variable	Reference range	Time (min)	Oral administration		IV administration	
			15 mg/kg	20 mg/kg	10 mg/kg	20 mg/kg
LY30 (%)	83.0–87.2	0	84.02 ± 3.77	68.25 ± 33.97	86.02 ± 4.62	84.40 ± 7.01
	—	60	45.82 ± 27.72*‡	37.67 ± 12.07*	34.15 ± 17.33*†	25.48 ± 16.28*
	—	240	25.08 ± 14.60*[a]	19.90 ± 19.40*[a]	53.45 ± 18.72*[b]	36.50 ± 18.05*[a,b]
	—	360	33.58 ± 22.73*[a,b]	20.90 ± 18.00*[b]	65.21 ± 18.61*[c]	40.87 ± 25.07*[a]
LY60 (%)	91.3–93.1	0	91.82 ± 1.89	76.47 ± 35.87	92.8 ±1.95	92.15 ± 3.21
	—	60	72.68 ± 15.27*	67.55 ± 7.94*†	65.65 ± 11.50*	58.23 ± 13.29*
	—	240	57.65 ± 14.31*[a,b]	47.5 ± 20.79*[a]	76.77 ± 10.62[b]	66.55 ± 14.00*[a]
	—	360	60.9 ± 21.24*[a,c]	47.60 ± 25.19*[a]	82.95 ± 9.08[b]	69.17 ± 14.00*[b,c]

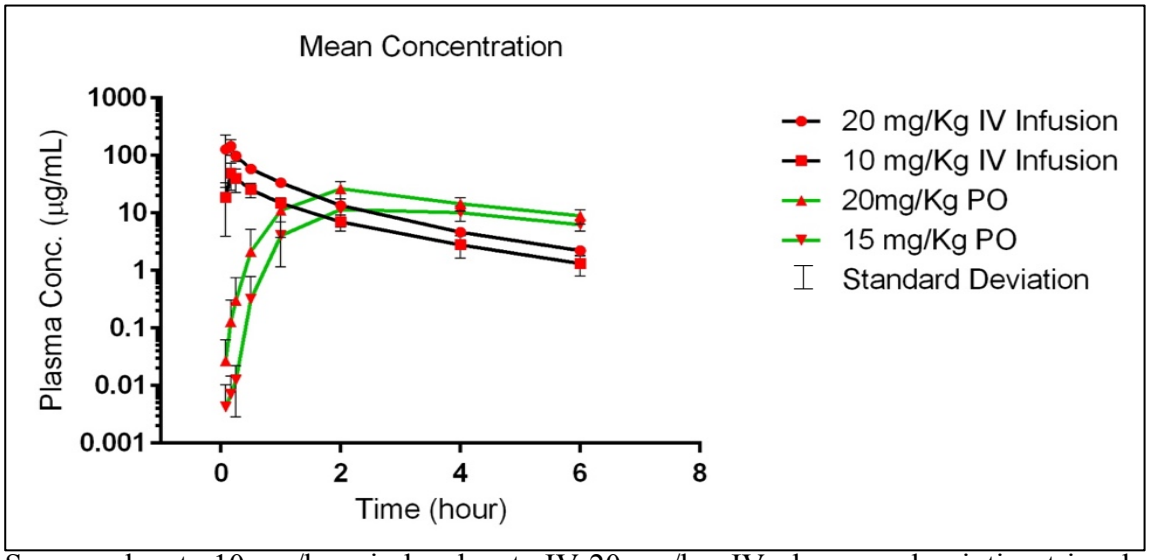
*Within a column, value differs significantly from value at 0 minutes. †Within a column, value differs significantly from value at 240 and 360 minutes. ‡Within a column, value differs significantly from value at 240 minutes. [a,b,c] Within a row, values with different superscript letters are significantly different.

Table 2.3: Mean ± SD pharmacokinetic parameter estimates for TXA following IV and oral administration to the same 6 dogs as in **Table 2.1**.

Parameter	**IV administration**		**Oral administration**	
	10 mg/kg	**20 mg/kg**	**15 mg/kg**	**20 mg/kg**
t_{max} (min)	14.2 ± 8.0	8.33 ± 2.58	144 ± 54	120 ± 0
C_{max} (µg/mL)	53.7 ± 17.6	181 ± 75	13.3 ± 3.7	26.5 ± 8.9
AUC_{0-last} ([µg•min]/[mL])	3,040 ± 490	7,080 ± 968	2,960 ± 890	5,210 ± 1,190
$AUC_{0-\infty}$ ([µg•min]/[mL])	3,230 ± 530	7,360 ±1,020	5,070 ± 1,590	7,580 ± 1,410
Vd (mL/kg)	398 ± 126	365 ± 37	—	—
Cl (mL/kg/min)	3.18 ± 0.62	2.76 ± 0.42	—	—
$t_{1/2}$ (min)	86.2 ± 19.8	92.1 ± 4.5	—	—

Bioavailability was not estimated for the oral doses because the predicted AUC data for the oral doses from the last collection time (6 hours) to infinity represented only 41.0 ± 13.7% and 30.7 ± 13.7% of the total estimated $AUC_{0-\infty}$ for the 15 and 20 mg/kg oral doses, respectively. Cl = Systemic clearance. — = Not applicable.

Figure 2.1—Plasma drug concentration-versus-time profiles for tranexamic acid in 6 healthy dogs that received each of the 4 treatments in a randomized, crossover-design study with a 1-week washout period between experiments.



Squares denote 10 mg/kg; circles denote IV 20 mg/kg, IV; downward-pointing triangles denote 15 mg/kg, PO; upward-pointing triangles denote 20 mg/kg, PO. Values are shown as mean ± SD.

Chapter 3

Pharmacokinetic Modeling and Simulation of Tranexamic Acid in Dogs

3.1. Introduction

Tranexamic acid (TXA) is a lysine analogue and anti-fibrinolytic drug. It competitively prevents plasminogen activation by binding to the lysine residues within the fibrin clot **(Figure 1.2)**. TXA is highly water soluble (167,000 mg/L) and is excreted as unchanged drug from the kidney.[59,93] The drug is used to stop bleeding and decrease the risk of bleeding complications. It is approved by the FDA for patients with hemophilia undergoing dental extractions and for females experiencing severe bleeding during menstruation. TXA is also used widely for bleeding management in surgical or injury related trauma. It has been shown to be clinically effective in decreasing mortality among patients with acute coagulopathy of trauma and shock (ACOTS). A bolus dose of TXA 10 mg/kg achieves plasma concentrations of 10μg/mL which has been shown to inhibit 80% of fibrinolysis *in vitro*, and a dose of 100 mg/kg was shown to inhibit fibrinolysis completely. However, the optimal TXA concentration to inhibit fibrinolysis completely *in vivo* is still unknown which could explain why there are several ongoing trials to optimize dosing in human clinical settings.[59]

As mentioned earlier in **Chapter 1**, dosing of TXA varies based on the type of surgery and the severity of bleeding. Patients undergoing cardiac surgeries typically

receive a loading dose of 30 mg/Kg (IV bolus) and a maintenance dose of 16 mg/Kg/hr (IV infusion), while low bleeding risk patients receive a loading dose of 10 mg/Kg (IV bolus) and 1 mg/Kg/hr (IV infusion) maintenance dose. Patients undergoing spinal surgeries typically receive a loading dose between 10-20 mg/kg (IV bolus), and maintenance doses ranging from 10 to 100 mg/Kg/hr (IV infusion). Finally, patients with trauma can receive a bolus dose of 1 g over 10 minutes followed by a maintenance dose of 1 g over 8 hours. However, lower dose of TXA 10 mg/Kg (IV bolus) followed by 1 mg/Kg/hr (IV infusion) effectively and safely reduced the risk of bleeding, while doses of 100 mg/kg and greater are associated with increased risk of seizures.[59]

Regardless to the different dosing strategies used in human, the evaluation of TXA in animals is still needed due to the growing use and importance of TXA in veterinary clinical settings. Although TXA underwent preclinical evaluation in animals, including dogs, the available literature is not sufficient to make dosing recommendations that are optimal for use in dogs. One study (Brained, 2012) showed that the required concentration to inhibit fibrinolysis in dogs was 144.7 μg/mL *in vitro*.[86] However, our recent data **(Chapter 2)** showed that nausea and vomiting were observed when *in vivo* systemic TXA concentrations were within the previously suggested level. In addition, lower plasma concentrations of TXA were able to maintain fibrin clot. Because of the differences in literature and potential for clinically meaningful toxicities or treatment failure, a greater understanding of TXA pharmacokinetics (PK) was needed. The goal of the research presented in this chapter was to develop a PK computational model using observed data (described in **(Chapter 2)** and perform simulations of different doses and dosing frequencies, as a first step towards optimization of TXA therapy in dogs. The results of this

study will help veterinary clinicians and investigators examine different dosing strategies that can be used to establish different exposure-response relationships of TXA in dogs, specifically its anti-fibrinolytic efficacy and observed toxicities, and develop optimal dosing schedules.

3.2. Methods

3.2.1. Study Design

Six healthy dogs were assigned to receive a single dose of TXA *via* a 10 minutes IV infusion through an indwelling jugular venous catheter (10 mg/kg or 20 mg/kg) or PO (15 or 20 mg/kg) in the form of a crushed tablet. The study was utilized a randomized, crossover design. Each dog received all treatments during the course of the study with a 7-day washout period between treatments. Food was withheld ≥ 12 hours prior to TXA administration. Blood samples for pharmacokinetic analysis (1 mL) were collected into EDTA-containing blood tubes at baseline (0) and at 5, 10, 15, 30, 60, 120, 240, and 360 minutes after drug administration. The EDTA-containing samples were held on ice until centrifugation (1,500 X *g* for 10 minutes at room temperature), which was performed ≤ 1 hour after collection. Plasma supernatant was removed and stored at –80 ^{o}C for ≤ 4 months until analysis. Samples were batch analyzed by high-performance liquid chromatography–mass spectrometry at a commercial toxicology laboratory using a method that was validated and met criteria established by the Food and Drug Administration.

3.2.2. Pharmacokinetic Analysis and Modeling

3.2.2.1. Non-compartmental Analysis

The pharmacokinetics parameters were estimated initially by a non-compartmental analysis from the plasma drug concentration–time profile data following a 10-minute IV infusion or oral administration using (WinNonlin Version 5.2, Pharsight Corp., Cary, NC). Parameters calculated from IV data included C_{max}, t_{max}, AUC_{0-last} (ie, the AUC from time 0 [drug administration] to 6 hours after administration), $AUC_{0-\infty}$, $t_{1/2}$, Vd, and systemic clearance. Parameters following oral drug administration included C_{max}, t_{max}, AUC_{0-last}, and $AUC_{0-\infty}$. The C_{max} and t_{max} were determined by examination of the drug concentration–time profiles, and AUC values were estimated by the log-trapezoidal method and the method for extrapolating exposure from the last measurement concentration to infinity.[94] A preliminary assessment of oral bioavailability was performed by comparing the oral $AUC_{0-\infty}$ /oral dose divided by IV $AUC_{0-\infty}$ /IV dose.

3.2.2.2. Pharmacokinetic Modeling

A pharmacokinetic two-compartment model (computational model) analysis with first-order elimination was performed using a nonlinear regression analysis program (WinNonlin Version 5.2, Pharsight Corp., Cary, NC). Individual animal data were analyzed using the naïve average of PK estimated parameter values obtained from the NCA as initial estimates. A two-compartment linear pharmacokinetic model (Appendix 1) was fitted to the each of the observed plasma TXA concentration-time profile for each using the following explicit PK equation:

$$C = Ae^{-\alpha t} + Be^{\beta t} \qquad \textbf{Equation 1.}$$

where C is the drug concentration in plasma, A and B are the mass constants that represents the ordinate intercepts for the distribution and elimination phases; α and β are the slopes of the distribution and elimination phases. Model fitting was discriminated based on goodness of fit (visual inspection), the Akaike's Information Criterion (AIC) and the sum of squares.[95,96] The resulting model was used to simulate drug concentration-time profile following several single short-term 10 minutes IV infusion doses and several IV bolus followed by long-term IV infusion doses.

3.3. Results

3.3.1. Pharmacokinetic Modeling

Figure 3.1 and **Figure 3.2** represent the pooled dog plasma drug concentration-time profiles after two IV 10 minutes infusion single doses of TXA (10 mg/Kg or 20 mg/Kg). TXA exhibited multi-exponential drug disposition and was best described by a linear two-compartment PK model with first order elimination (**Appendix 1**).

The average of total observed data from the non-compartmental analysis in **Chapter 2** was used to obtain initial PK parameter estimates. Two methods were used to generate predictions and predicted estimates. A naïve two-stage analysis using the average of individual predictions **(Figures 3.15** and **3.16** and average estimated values were generated after independently fitting the model to each individual data set for both the 10 mg/Kg dose and 20 mg/Kg IV doses. PK parameter estimates following the two-stage analysis show that the maximum concentration (C_{max}) for dogs that received the 10 mg/Kg IV dose was achieved right after the end of infusion at 10 minutes, except for two dogs. One achieved C_{max} at 15 minutes and the other dog, which was excluded from the analysis,

achieved C_{max} at 30 minutes. For the 20 mg/Kg IV dose, two dogs reached their C_{max} within the first 5 minutes. All other dogs reached their C_{max} at 10 minutes **(Table 3.1)**. **Figure 3.5** and **Figure 3.6** represents the average of the predicted data and average of the observed drug concentration-time profiles from 5 of the 6 dogs received the 10 mg/Kg IV dose and all 6 dogs that received the 20 mg/Kg IV dose.

A mean analysis was also used for PK modeling by fitting the model to the average of total observations of the 20 mg/Kg IV doses. The PK estimates of the mean analysis are listed in **Table 3.2**. **Figure 3.17** represents average of the observed data, predicted data of the average observations and simulated data concentration-time profiles of the 20 mg/Kg IV dose. Fitting of the model was achieved after curve weighting by inverse of the model observed drug concentration (1/y) for the 10 mg/Kg IV dose, and curve weighting by inverse of the square model observed drug concentration ($1/y^2$) for the 20 mg/Kg IV dose.

3.3.2. Pharmacokinetic Simulations

3.3.2.1. Short-term 10 minutes IV infusion

Dosing simulations were performed for 5, 10, 20, 40 and 80 mg/Kg doses **(Figure 3.18)** by assuming linear and stationary kinetics, fixing PK parameters, based on the mean analysis PK model estimated values of the 20 mg/Kg IV dose. The simulated data of the 20 mg/Kg IV dose matched the predicted data from the model **(Figure 3.17)**. The 10 mg/kg simulation accurately predicted the observed data of the 10 mg/Kg IV **(Figure 3.18)**.

3.3.2.2. Loading dose (IV bolus) followed by long-term maintenance dose (IV infusion)

Model simulation of the 20 mg/Kg (IV bolus) followed by 2 mg/Kg/hr (IV infusion), 15 mg/Kg (IV bolus) followed by 2 mg/Kg/hr (IV infusion), and 10 mg/Kg (IV bolus) followed by 2 mg/Kg/hr (IV infusion) predicted steady state TXA concentrations of approximately 15 µg/mL, which were achieved after three hours. Simulations of the loading doses 20 mg/Kg and 15 mg/Kg IV bolus achieved C_{max} of 200 µg/mL and 150 µg/mL, respectively. Simulated data of 10 mg/Kg (IV bolus) and 5 mg/Kg (IV infusion) showed that predicted steady state concentration of 30 µg/mL was achieved within the first hour. Simulated data of 10 mg/Kg (IV bolus) and 1 mg/Kg (IV infusion) showed that a predicted plasma concentration of approximately 10 µg/mL was achieved after 2 hours. All simulated data were generated based on the mean analysis PK model estimated values of the 20 mg/Kg IV dose **(Table 3.3** and **Figure 3.19)**.

3.4. Discussion

A two-compartment PK model (computational model) was developed for TXA following 10 minutes IV infusion. The PK parameter estimated from the model were similar to the PK values obtained from the non-compartmental analysis (NCA) **(Chapter 2)**. Average C_{max} of the 20 mg/Kg dose was three times greater than the 10 mg/Kg dose because of the variability in the infusion, which could be related to improper drug injection resulted in the variation of the infusion rate since two of the dogs that received the 20 mg/Kg dose achieved C_{max} at 5 minutes and one dog that received a 10 mg/Kg dose

achieved a C_{max} at 15 minutes. Another dog received 10 mg/Kg dose achieved C_{max} of 20 μg/mL at 30 minutes was excluded from the analysis. For this particular dog, 30 minutes to achieve C_{max} could be related to a drug extravagation to the tissues around the jugular vein. However, the PK estimated values suggested that the drug follows linear kinetics within the range of the given doses since Cl, Vd and AUC/dose were not significantly different. This is in agreement with the literature for various doses of TXA in different species.

In the BART trial (Blood Conservation Using Antifibrinolytics in a Randomized Trial), a loading dose of 30 mg/Kg followed by 16 mg/Kg IV infusion was used during cardiac surgeries and achieved plasma concentrations greater than 100 μg/mL.[66] The goal of maintaining plasma TXA concentrations greater than 100 μg/mL was to achieve complete fibrinolysis inhibition.[66] However, human studies showed that these concentrations of TXA increased risk of seizure.[59] In an *in vitro* model of corticoid slices, concentrations of 30 μg/mL induced the excitatory pathway which has been associated with seizures.[97] Additionally in **Chapter 2**, the IV dose of 20 mg/Kg achieved drug concentrations greater than 100 μg/mL which induced nausea and vomiting in dogs. This would suggest plasma drug concentrations greater than 100 μg/mL are associated with additional adverse events.

A dosing regimen of 1 g and 1 g over 8 hours for patients with trauma was used in the CRASH-2 trial (Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage 2 trial).[62] Assuming that average human body weight is about 70 Kg, a loading dose of 15 mg/Kg (IV bolus) and a maintenance dose of 2 mg/Kg (IV infusion) would be equal to the dosing regimen used in the trial. [62] The simulated data of this dose in dogs

achieved a steady state concentrations of approximately 15 μg/mL, which was within the TXA therapeutic level in human.[97]

In pediatric patients undergoing craniosynostosis surgeries, a two-compartment model analysis of TXA of a 10 mg/Kg loading dose (IV bolus) followed by a maintenance dose of 5 mg/kg/hr (IV infusion) resulted in a TXA concentration of 16 μg/mL.[98] Our analysis showed that this dossing schedule achieved and maintained plasma TXA concentrations of 30 μg/mL which was double of the concentrations observed in the plasma of pediatric patients that received the same dose. This would suggest that dogs have slower elimination rates than pediatric human patients.

Although our simulations matched the observed data of the 10 mg/kg IV 10 minutes IV infusion dose, the simulated dosing regimens have not been fully validated and examined which is one of the limitations of our model. Another limitation is that the drug has only been tested in healthy dogs using an *in vitro* model of fibrinolysis. Evaluation of the drug in dogs with coagulopathy would give better correlations between the drug exposure and the response. In **Chapter 2**, the bioavailability exceeded 100% which could be due to administering crushed and divided tablets (to achieve indicated dose/kg) resulting in misdoing or AUC overestimations, since the average missing portions of AUC were between 30% to 50% limiting our ability to accurately fit the model to oral data. Further the acquisition of additional data points beyond 6 hours would likely improve the overall estimate of exposure (AUC) following PO dosing schedules.

Table 3.1: Pharmacokinetic parameters obtained by computational modeling analysis after TXA single IV infusion doses (10 mg/Kg, 20 mg/Kg).

Pharmacokinetic Parameter Estimates	10mg/Kg IV		20mg/Kg IV	
	Estimated values	CV%	Estimated value	CV%
C_{max} (µg/mL)	50	12	135	14
Clearance (mL/min)/Kg	3.2	261	3	8
Distribution Half-life (min)	43	71	15	38
Terminal Half-life (hr)	4	1043	1.5	19
Elimination Half-life K10 (min)	44	265	29	21
Vd mL/kg	183	15	127	21
AUC $_{0-\infty}$ (µg /mL*hr)	54.5	260	112	8

A two-compartment model with first order elimination was fitted to the individual observed data of each dog and was used to estimate these parameters (two-stage analysis).

Table 3.2: Pharmacokinetic parameters obtained by computational modeling analysis after TXA single IV infusion doses (20 mg/Kg).

Parameter	20mg/Kg IV	
	Value	CV%
C_{max} (µg/mL)	154	15
Clearance (mL/min)/Kg	3	9
Distribution Half-life (min)	9	39
Terminal Half-life (hr)	1.4	16
Elimination Half-life K10 (min)	23	23
Vd (mL)/kg	98	22
AUC $_{0-\infty}$ (µg/mL*hr)	113	9

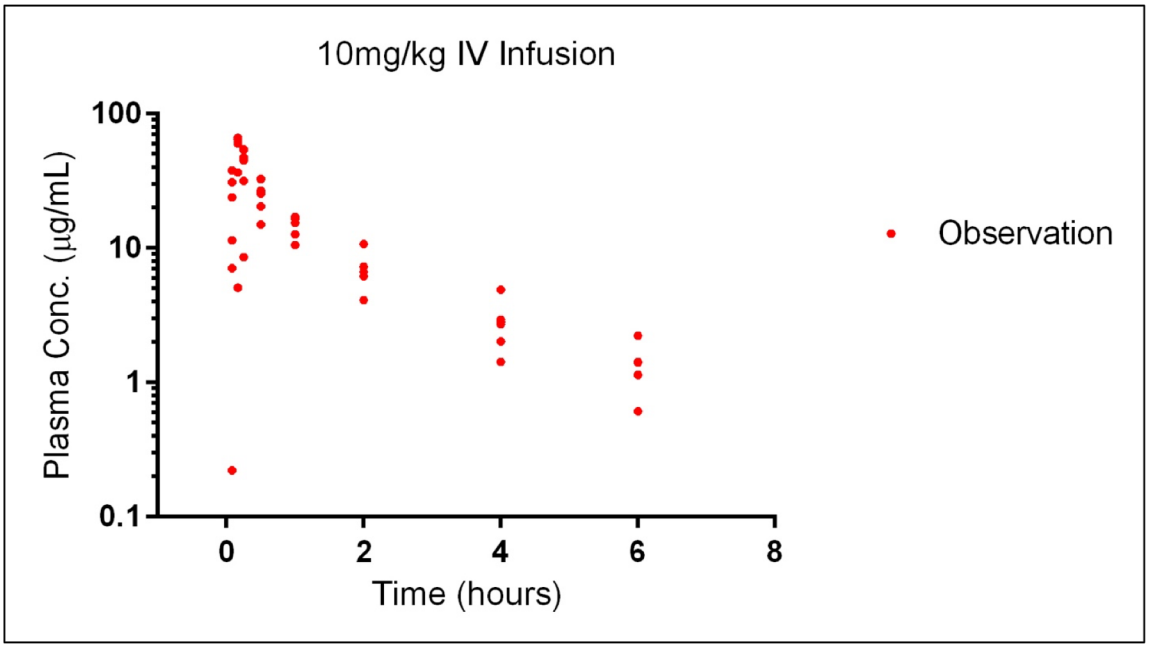
A two-compartment model with first order elimination was fitted to the averaged observed data (mean analysis) and was used to estimate these parameters.

Table 3.3: Simulated plasma concentrations in µg/mL at different time points after different TXA IV bolus loading doses (LD) followed by IV infusion maintenance doses (MD).

Dosing Schedule	Simulated Plasma Concentrations			
	C_{max} (Time zero)	First hour	Second hour	Third Hour
LD: 20 mg/Kg & MD: 2 mg/Kg/hr	200	30	20	16
LD: 15 mg/Kg & MD: 2 mg/Kg/hr	150	25	20	16
LD: 10 mg/Kg & MD: 2 mg/Kg/hr	100	17	16	14
LD: 10 mg/Kg & MD: 5 mg/Kg/hr	100	30	30	30
LD: 10 mg/Kg & MD: 1 mg/Kg/hr	100	15	10	9

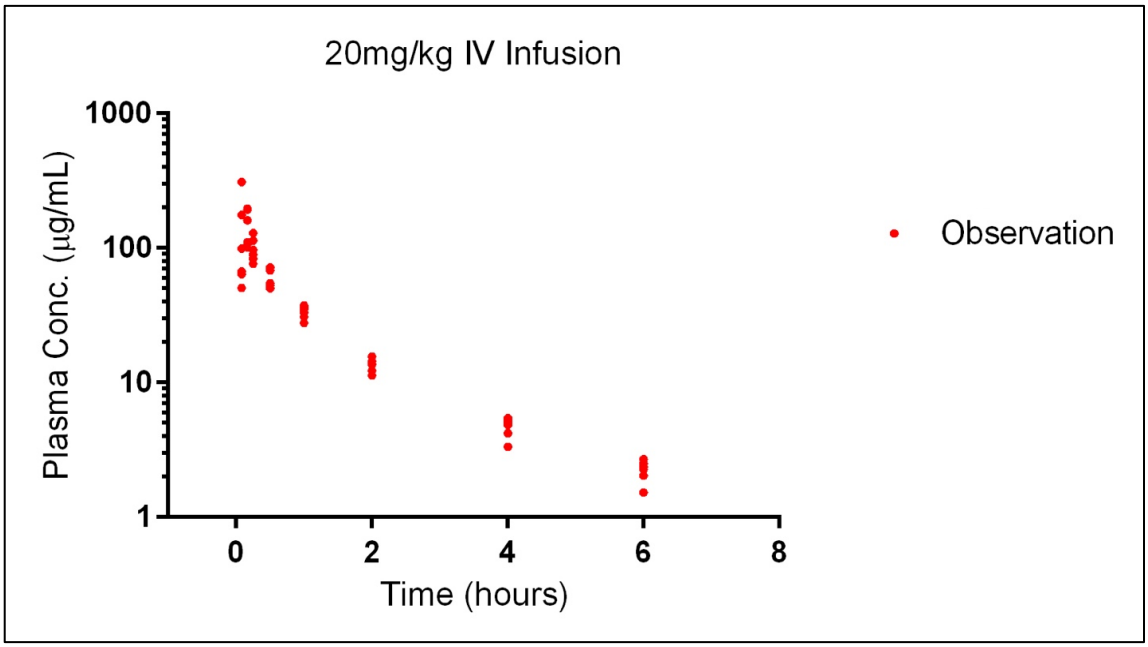
A two-compartment model with first order elimination was fitted to the averaged observed data of 20 mg/Kg IV 10 minutes infusion dose (mean analysis) and was used to generate simulated data.

Figure 3.1: Observed concentration-time PK profile of TXA 10 mg/Kg10 minutes IV infusion in 6 healthy dogs (pooled data).



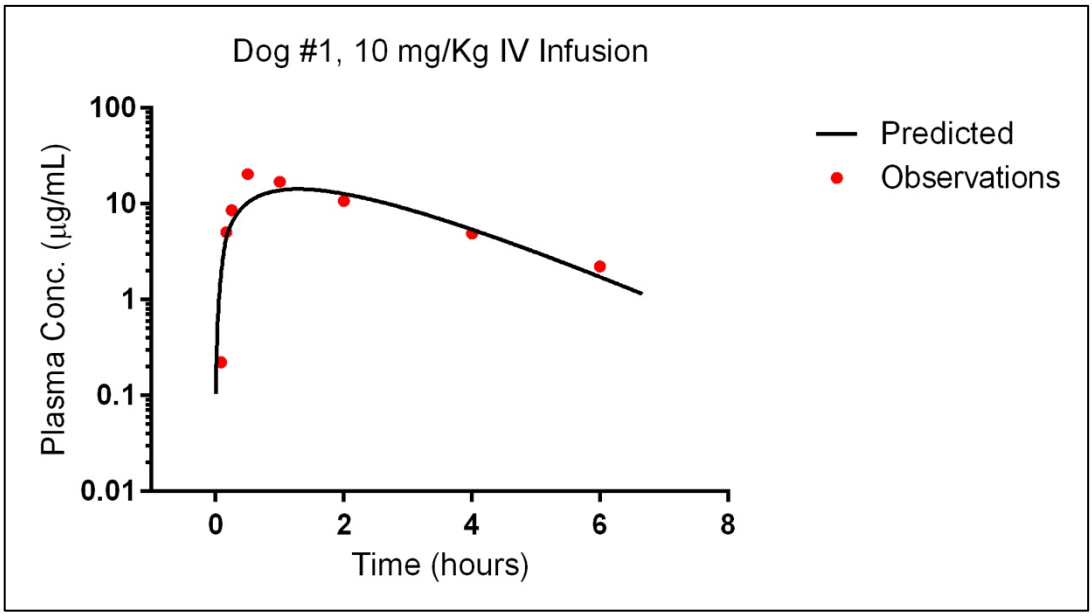
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); red circles denote observed concentrations data.

Figure 3.2: Observed concentrations-time PK profile of TXA 20 mg/Kg 10 minutes IV infusion in 6 healthy dogs (pooled data).



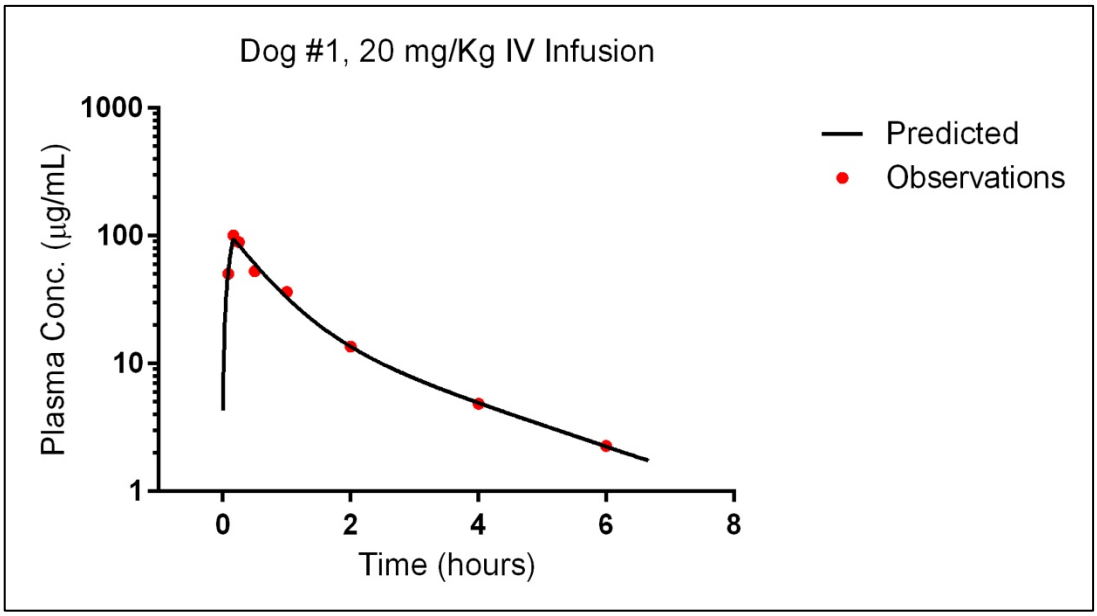
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); red circles denote observed concentrations data.

Figure 3.3: Predicted and observed concentration-time PK profiles of TXA 10 mg/Kg IV 10 minutes infusion in a healthy dog.



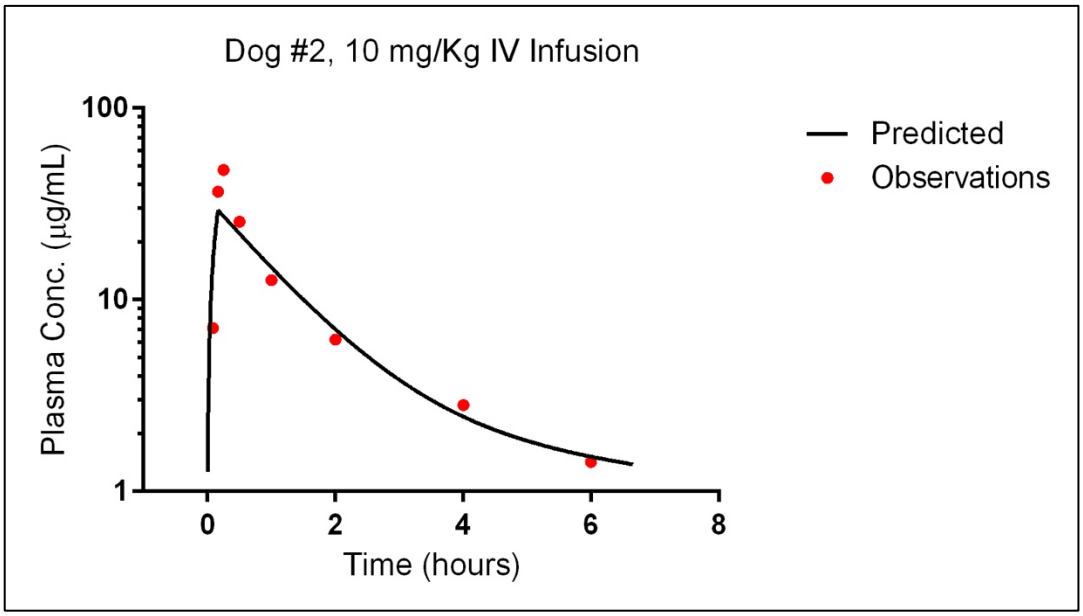
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.4: Predicted and observed concentration-time PK profiles of TXA 20 mg/Kg IV 10 minutes infusion in a healthy dog.



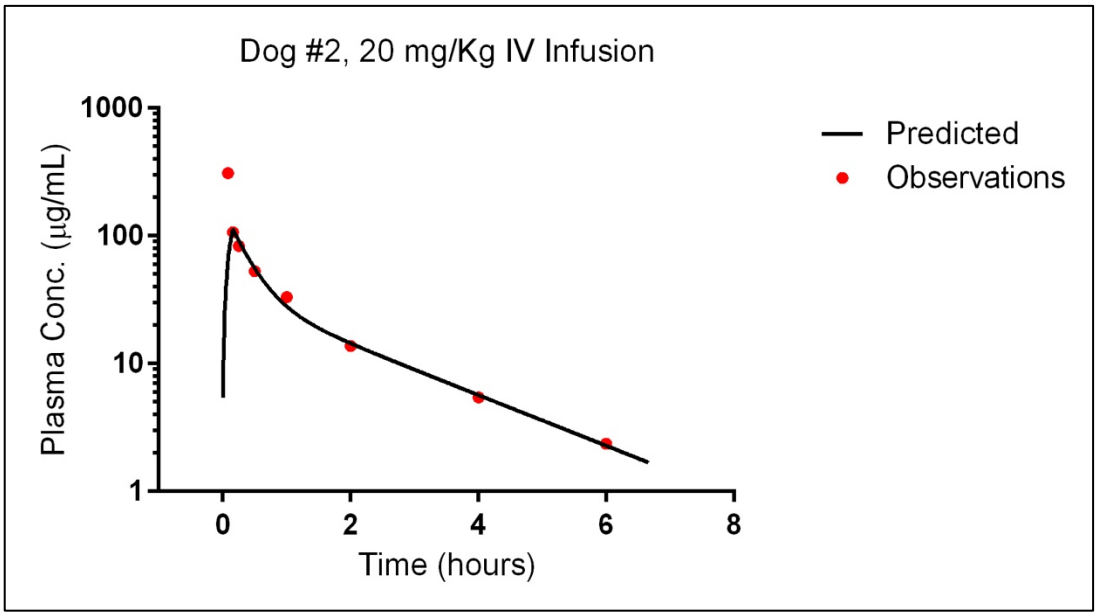
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.5: Predicted and observed concentration-time PK profiles of TXA 10 mg/Kg IV 10 minutes infusion in a healthy dog.



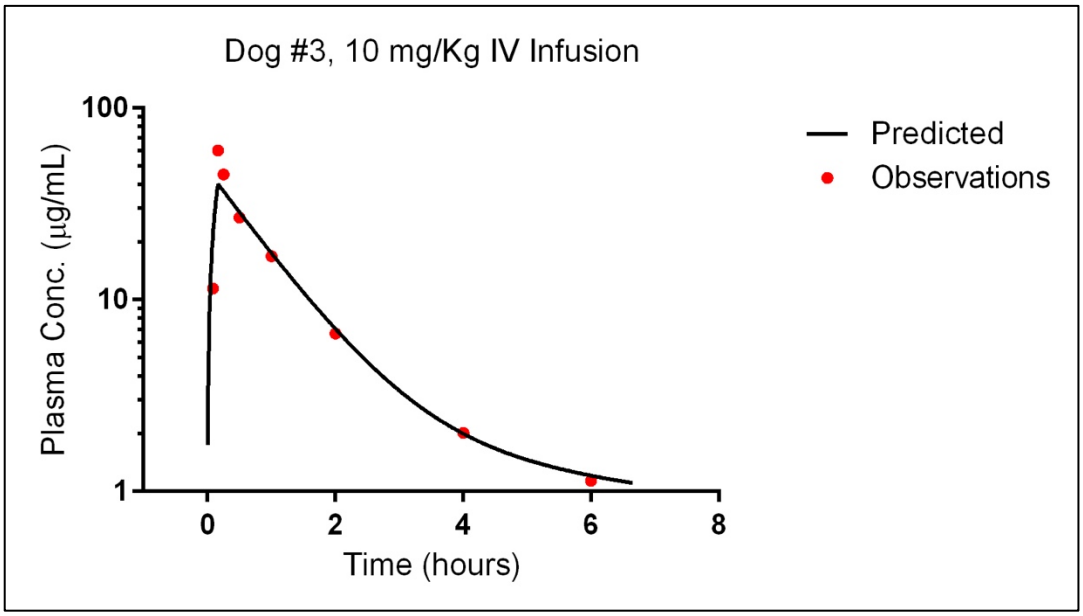
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.6: Predicted and observed concentration-time PK profiles of TXA 20 mg/Kg IV 10 minutes infusion in a healthy dog.



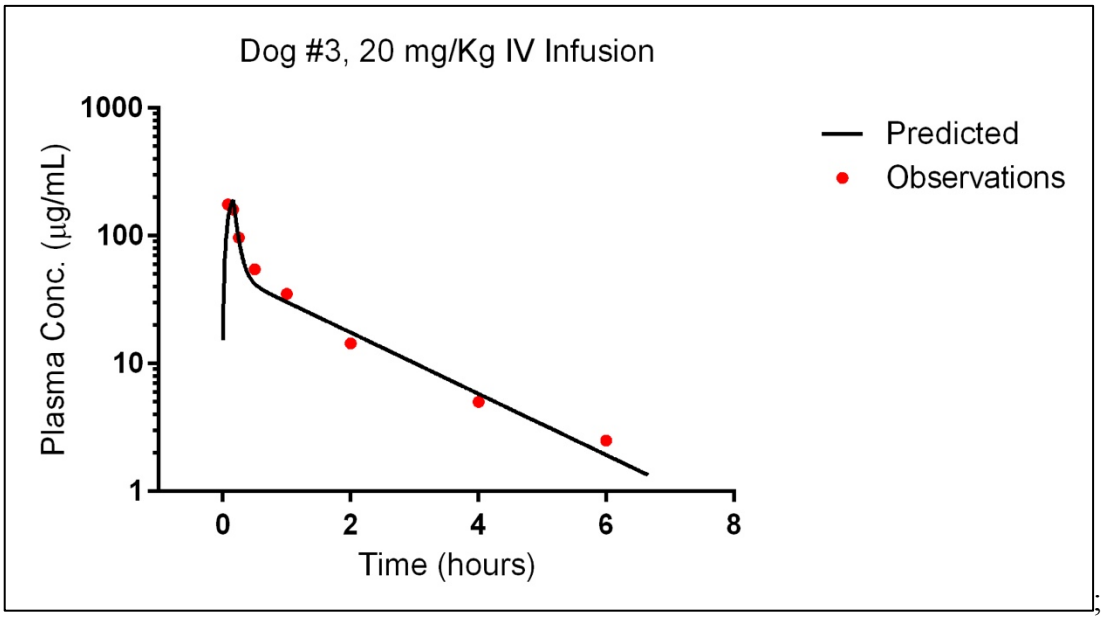
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.7: Predicted and observed concentration-time PK profiles of TXA 10 mg/Kg IV 10 minutes infusion in a healthy dog.



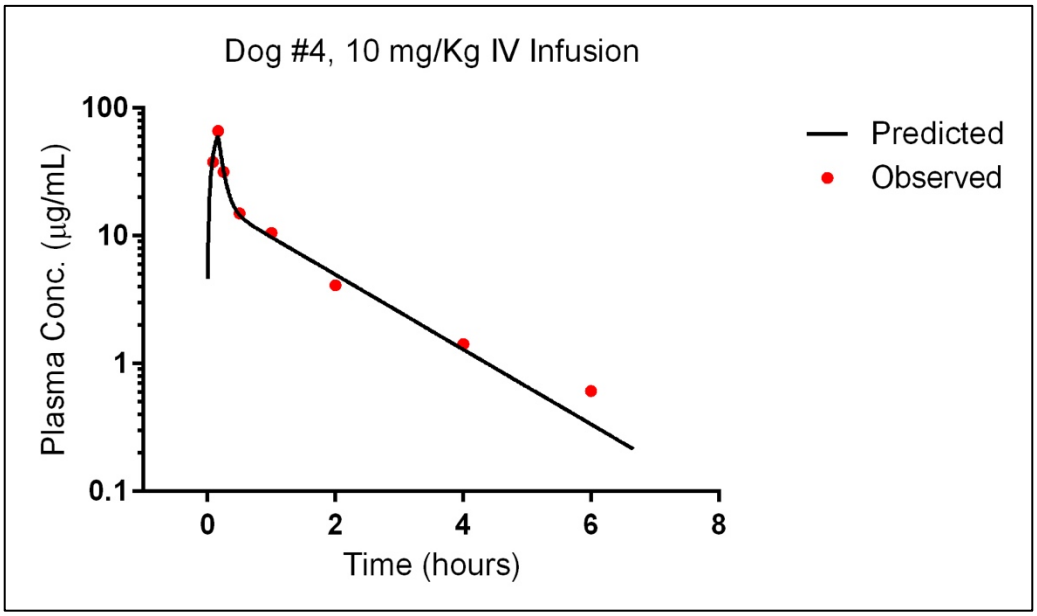
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.8: Predicted and observed concentration-time PK profiles of TXA 20 mg/Kg IV 10 minutes infusion in a healthy dog.



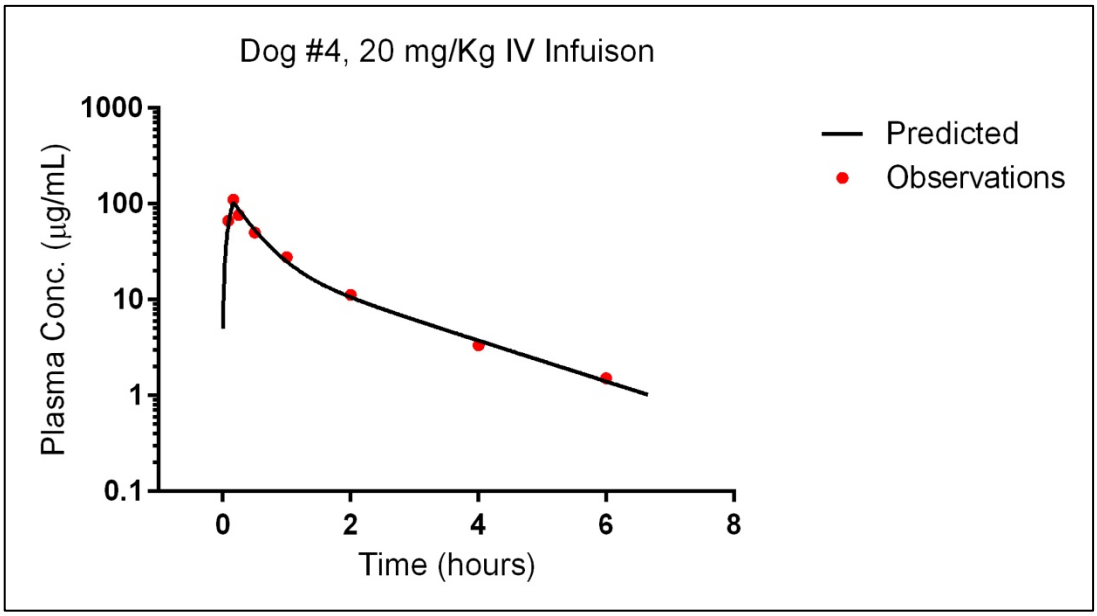
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.9: Predicted and observed concentration-time PK profiles of TXA 10 mg/Kg IV 10 minutes infusion in a healthy dog.



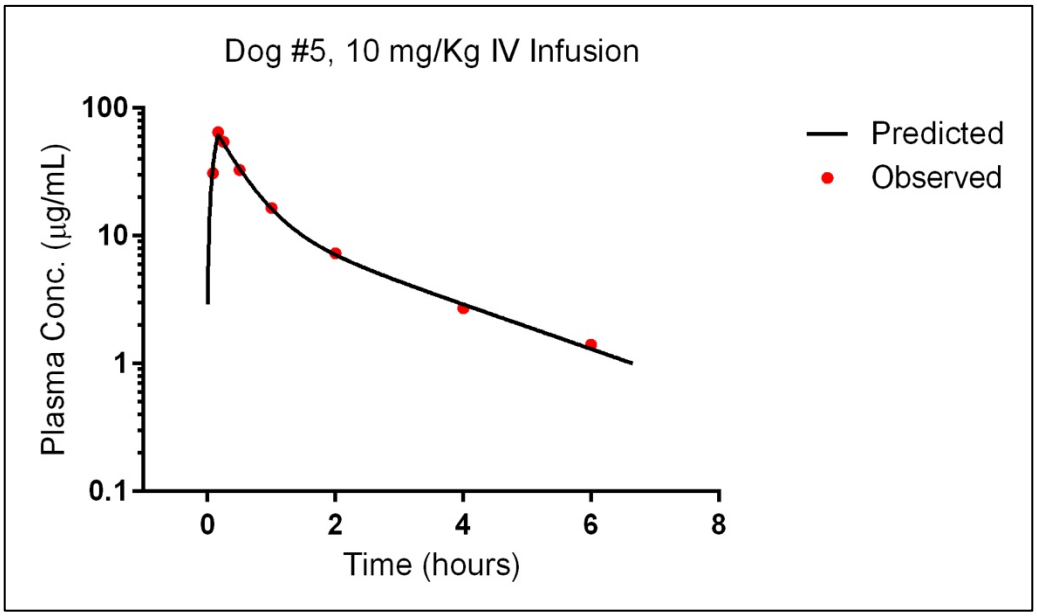
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.10: Predicted and observed concentration-time PK profiles of TXA 20 mg/Kg IV 10 minutes infusion in a healthy dog.



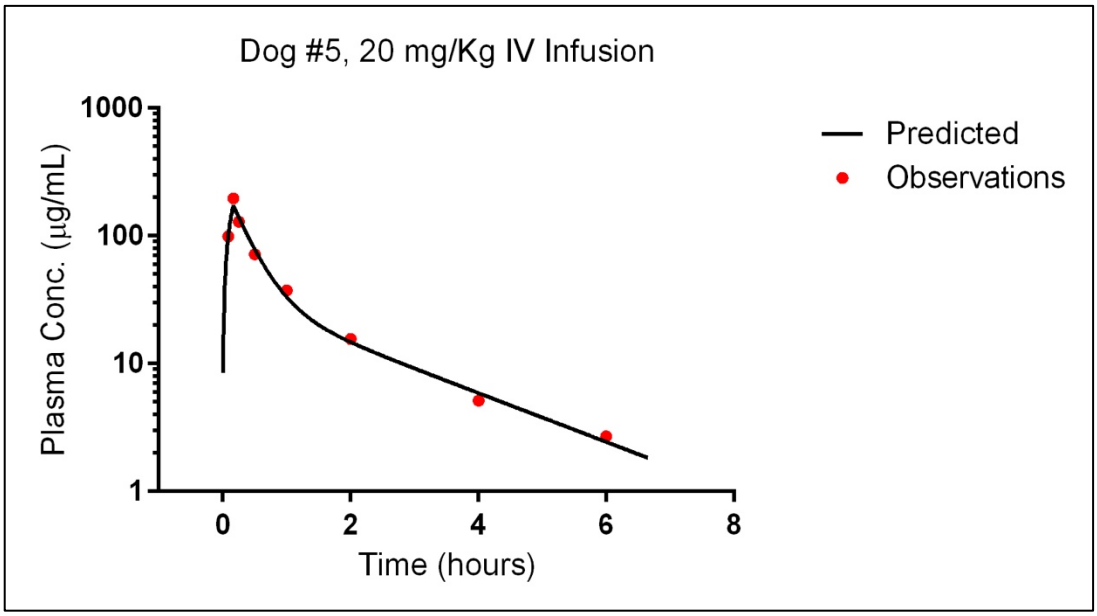
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.11: Predicted and observed concentration-time PK profiles of TXA 10 mg/Kg IV 10 minutes infusion in a healthy dog



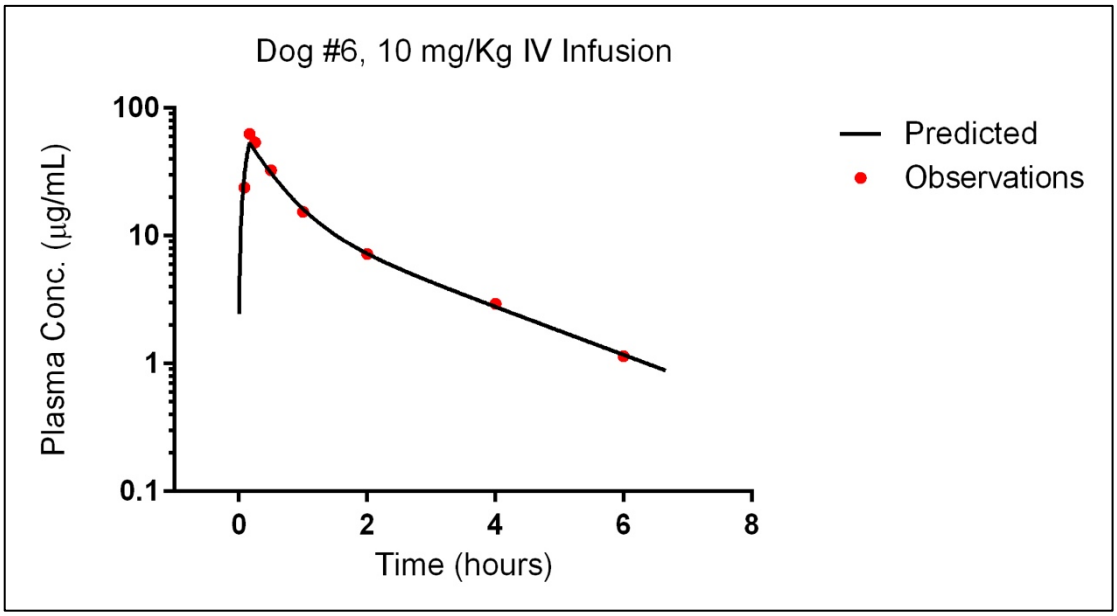
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.12: Predicted and observed concentration-time PK profiles of TXA 20mg/Kg IV 10 minutes infusion in a healthy dog.



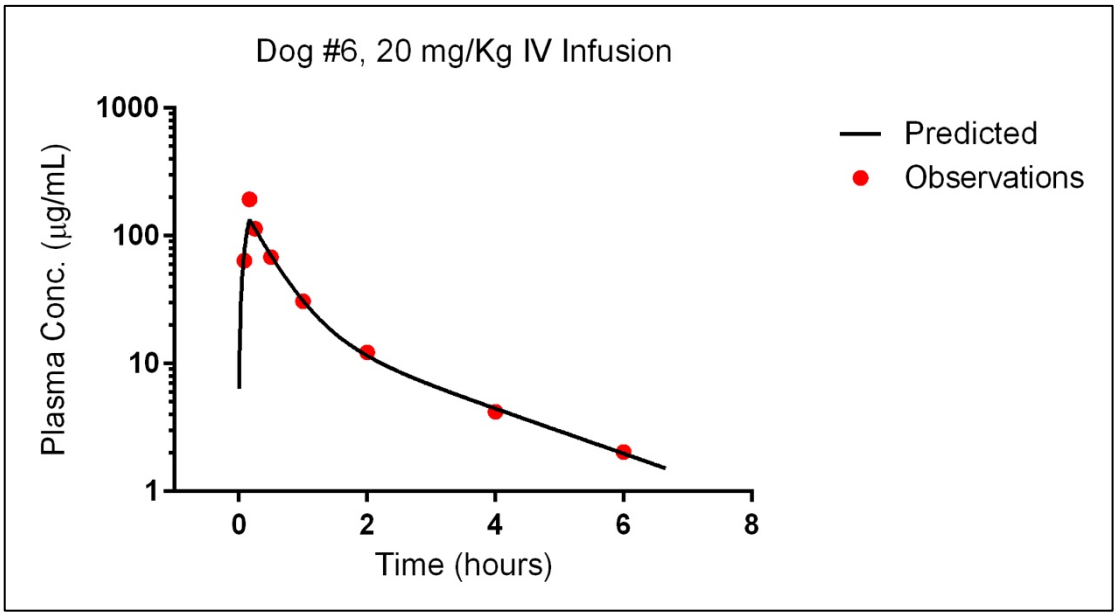
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure: 3.13: Predicted and observed concentration-time PK profiles of TXA 10 mg/Kg IV 10 minutes infusion in a healthy dog.



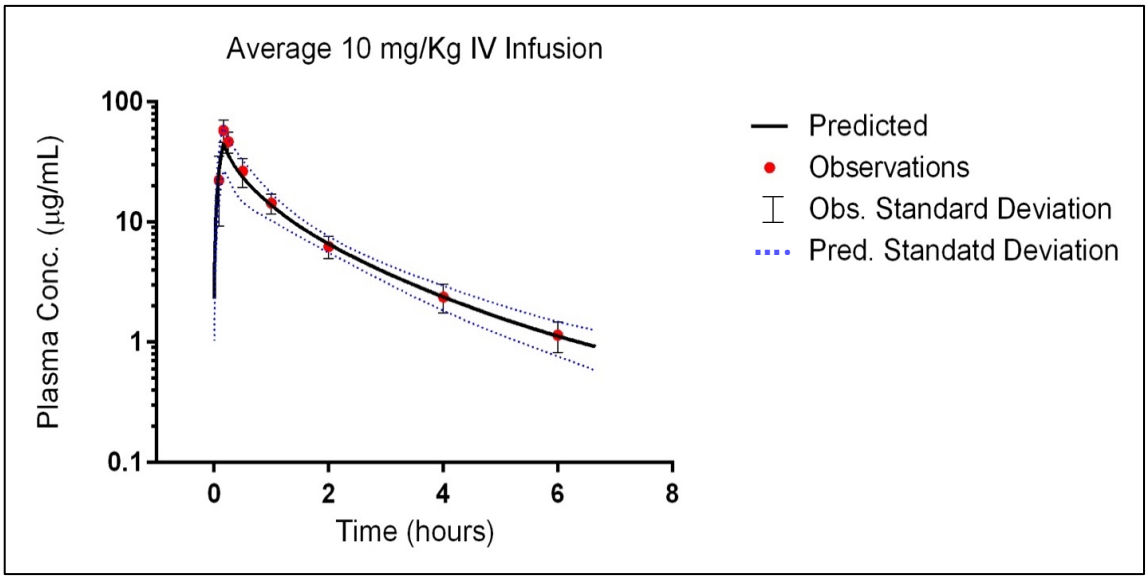
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.14: Predicted and observed concentration-time PK profiles of TXA 20 mg/Kg IV 10 minutes infusion in a healthy dog.



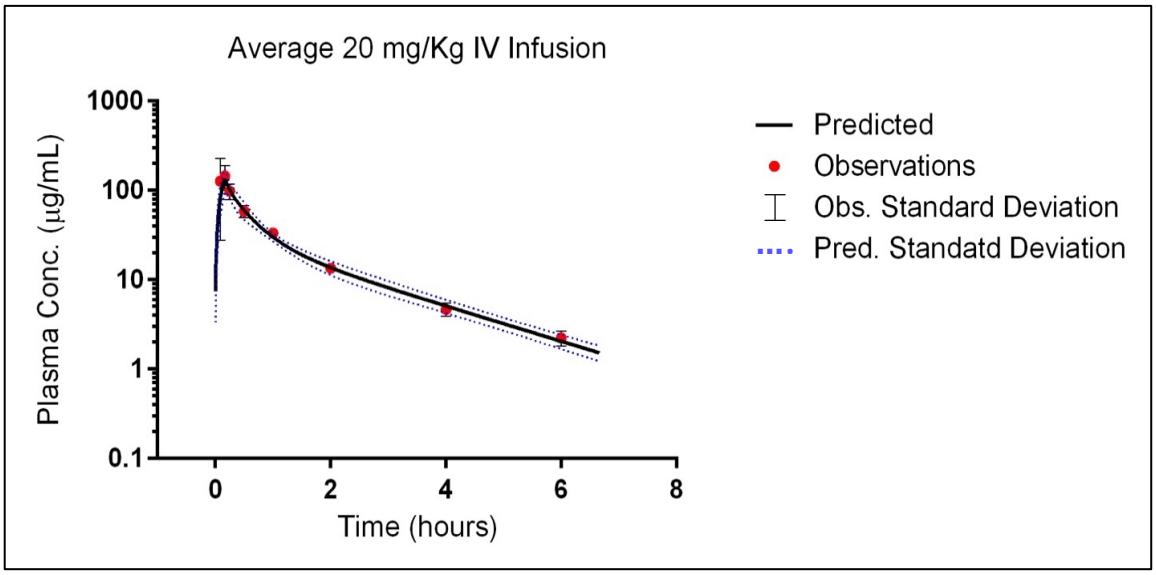
Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted concentrations data; red circles denote observed concentrations data; # denotes the identification number of the dog in the study. A two-compartment model with first order elimination was fitted to the individual observed data and was used to generate predictions.

Figure 3.15: Average predicted, and average observed plasma concentration-time PK profiles of TXA 10 mg/Kg IV 10 minutes infusion in 5 healthy dogs.



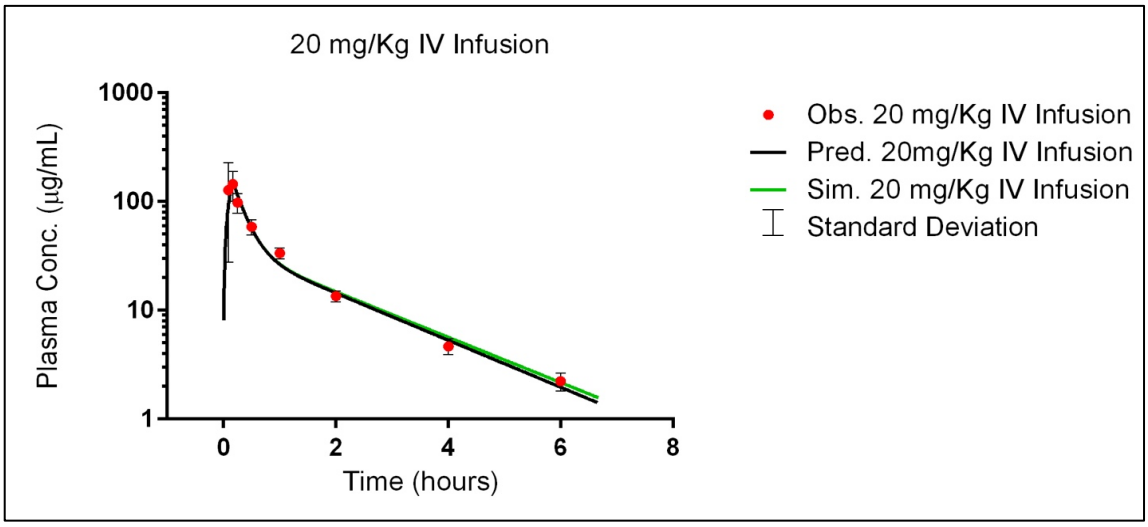
Y-axis denotes plasma concentration in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes average of the predicted data from the models; blue-dotted line denotes standard deviation from the mean of the averaged predicted data; red circles denote average of the observed data; ⌶ denotes standard deviation from the mean of the averaged observed data. A two-compartment model with first order elimination was fitted to the individual observed data of 10 mg/Kg IV 10 minutes infusion dose of each dog and was used to get the average of the predictions in 5 healthy dogs (two-stage analysis).

Figure 3.16: Average predicted and average observed plasma concentration-time PK profiles of TXA 20 mg/Kg IV 10 minutes infusion in 6 healthy dogs.



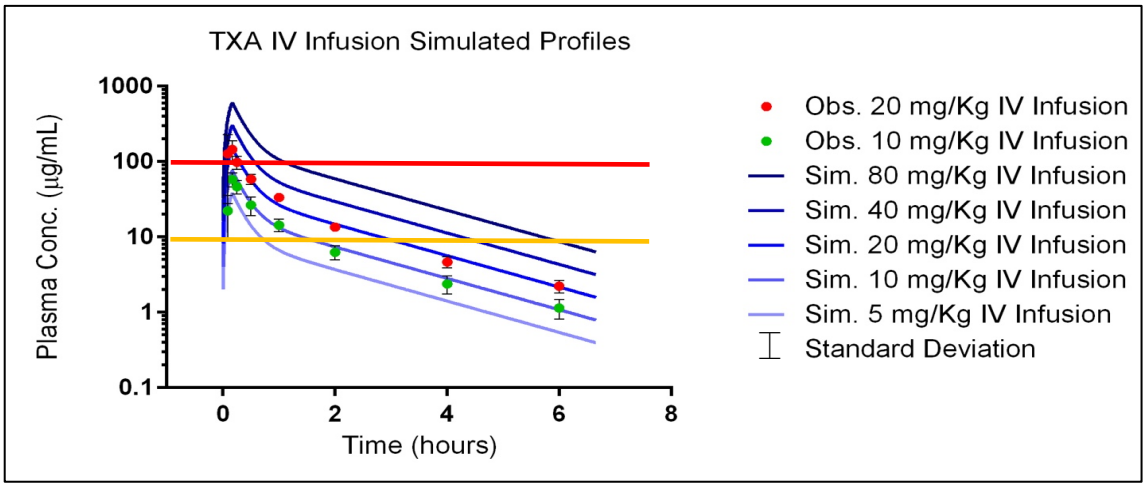
Y-axis denotes plasma concentration in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes average of the predicted data from the models; blue-dotted line denotes standard deviation from the mean of the averaged predicted data; red circles denote average of the observed data; ⌶ denotes standard deviation from the mean of the averaged observed data. A two-compartment model with first order elimination was fitted to the individual observed data of 20 mg/Kg IV 10 minutes infusion dose of each dog and was used to get the average of the predictions in 6 healthy dogs (two-stage analysis).

Figure 3.17: Simulated, predicted, and average observed plasma concentration-time PK profiles of TXA 20 mg/Kg IV 10 minutes infusion in 6 healthy dogs.



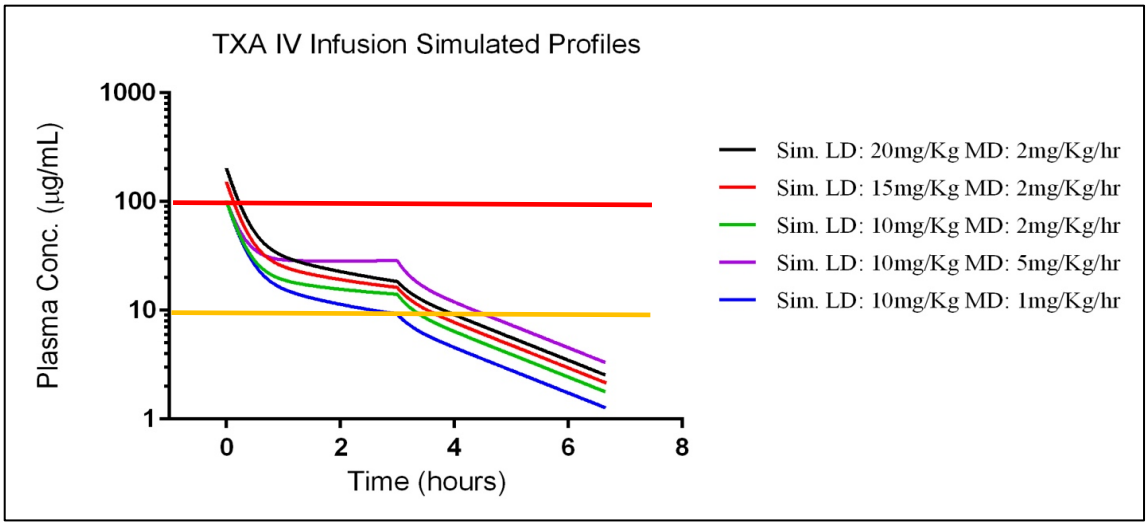
Y-axis denotes plasma concentration in logarithmic scale (µg/mL); X-axis denotes time (hours); black line denotes predicted data from the model; green line denotes simulated data; red circles denote average of observed data; ⌶ denotes standard deviation from the mean of the averaged observed data. A two-compartment model with first order elimination was fitted to the averaged observed data of 20 mg/Kg IV 10 minutes infusion dose (mean analysis) and was used to generate simulated data.

Figure 3.18: Simulated, predicted, and average observed plasma concentration-time PK profiles of TXA 5, 10, 20, 40, 80 mg/Kg IV 10 minutes infusion doses in healthy dogs.



Y-axis denotes plasma concentration in logarithmic scale (µg/mL); X-axis denotes time (hours); first line from the top (the darkest in color) denotes simulated data of 80 mg/Kg IV dose; second line from the top denotes simulated data of 40 IV mg/Kg dose; the third line in the middle denotes simulated data of 20 IV mg/Kg dose; the fourth line from the top denotes simulated data of 10 IV mg/Kg dose; the fifth line from the top (lightest in color) denotes simulated data of 5 mg/Kg IV dose; green circles denote averaged data from 5 healthy dogs received 10 mg/Kg IV 10 minutes infusion dose; red circles denote averaged data from 6 healthy dogs received 20 mg/Kg IV 10 minutes infusion; ⌶ denotes standard deviation from the mean of the averaged observed data; red line at 100 µg/mL denotes minimum toxic concentrations in human; yellow line at 10 µg/mL denotes minimum therapeutic concentration in human. A two-compartment model with first order elimination was fitted to the averaged observed data of 20 mg/Kg IV 10 minutes infusion dose (mean analysis) and was used to generate simulated data.

Figure 3.19: Simulated plasma concentration-time pharmacokinetic PK profiles in healthy dogs of a TXA loading dose (IV bolus) followed by a maintenance dose (IV infusion). Different doses were simulated.



Black line denotes TXA 20 mg/Kg IV bolus loading dose followed by 2 mg/Kg/hr IV infusion maintenance dose; red line denotes TXA 15 mg/Kg IV bolus loading dose followed by 2 mg/Kg/hr IV infusion maintenance dose; green line denotes TXA 10 mg/Kg IV bolus loading dose followed by 2 mg/Kg/hr IV infusion maintenance dose; violet line denotes TXA 10 mg/Kg IV bolus loading dose followed by 5 mg/Kg/hr IV infusion maintenance dose' blue line denotes TXA 10 mg/Kg IV bolus loading dose followed by 1 mg/Kg/hr IV infusion maintenance dose; Y-axis denotes plasma concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); red line at 100 µg/mL denotes minimum toxic concentrations in human; yellow line at 10 µg/mL denotes minimum therapeutic concentration in human. A two-compartment model with first order elimination was fitted to the averaged observed data of 20 mg/Kg IV 10 minutes infusion dose (mean analysis) and was used to generate simulated data.

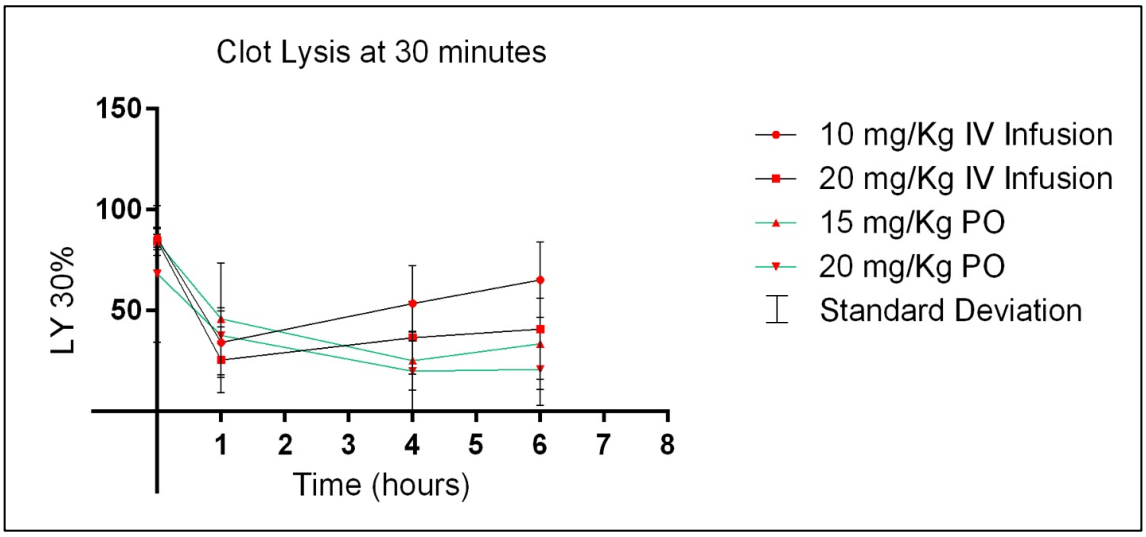
Chapter 4

Conclusion and Future Directions

The overall goal of this research focused on determining the pharmacokinetics (PK) of tranexamic acid (TXA) in dogs following short-term 10 minutes IV infusions and oral doses. Dose optimization of TXA has always been a clinical challenge in human and veterinary medicine. Many dosing regimens have been optimized for use in human, but there is a lack of similar studies investigating the exposure-response relationship of TXA in animals, specifically dogs. While this is not surprising, the growth of its clinical use in veterinary species necessitated further examination. **Chapter 1** reviewed and highlighted the effect and the use of variety of clinically approved drug agents targeting coagulation and fibrinolysis to gain a thorough understanding of the effect of different drug agents on clot formation. The chapter also discussed several bleeding disorders and the benefit of using lysine-analogue antifibrinolytics in decreasing the mortality and the risk of bleeding which led us to evaluate the use of TXA in dogs. In **Chapter 2**, *in vivo* studies were performed, and PK parameters were estimated following a non-compartmental analysis of 10 mg/Kg and 20 mg/Kg IV 10 infusion doses, and 15 mg/kg and 20 mg/kg oral doses received by six healthy dogs in a cross-sectional design. TXA significantly decreased the clot lysis percentage at 30 minute after the maximum amplitude (LY30) in dogs for all of the four doses. However, the IV doses effect did not last longer, and the effect of TXA after receiving the oral doses was delayed **(Figure 4.1)**. In **Chapter 3**, a PK computational

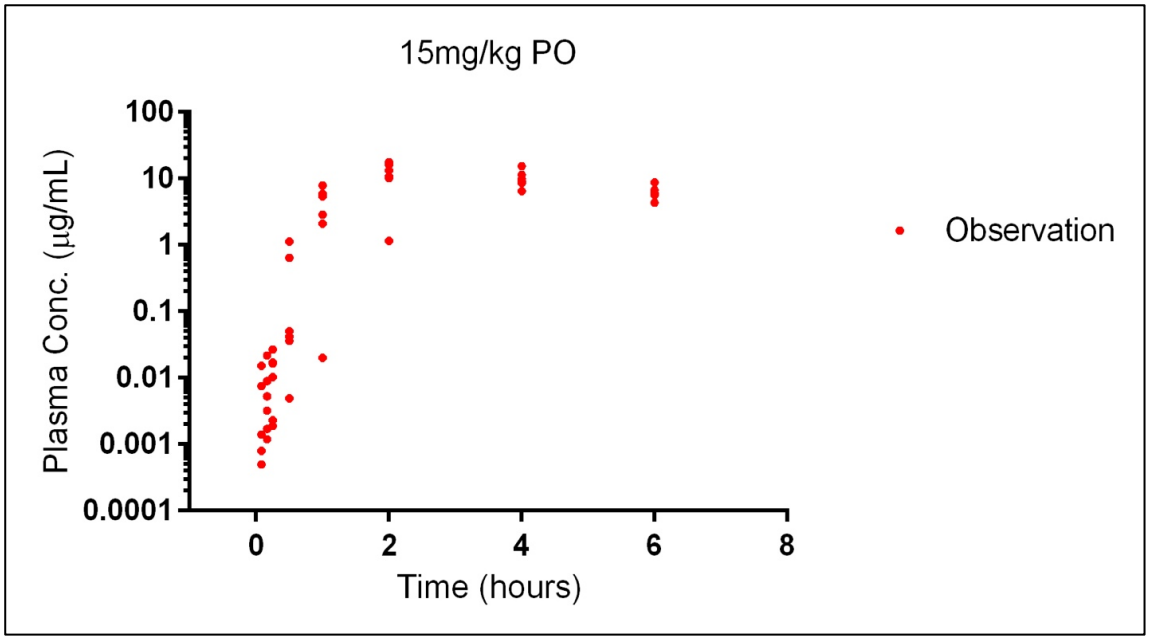
modelling and simulations were applied to examine and develop dosing regimens for dogs and to achieve therapeutic plasma concentrations in surgical and non-surgical settings. In the future, modelling the pharmacodynamic (PD) data are needed to correlate exposure with therapeutic and toxicological responses. Further studies in dogs will be needed to validate the developed model and to test the suggested dosing regimens. This will be beneficial as this will allow the examination and evaluation of various dosing regimens and allow for further model refinement. In addition, understanding the PK parameters of TXA oral doses requires modelling of the oral PK data. Integration of the PK values obtained from TXA IV model into an oral model might be beneficial to better capture the missing portions of the oral data profiles **(Figure 4.2** and **Figure 4.3)**. As mentioned in **Chapter 2**, the missing portions of the area under the time-concentration curves (AUC) were between 30% and 50%. Further experiments of the oral doses in dogs might be required to get more precise PK values related to the oral dosing. Finally, studying the effect of TXA in dogs undergoing surgeries or experiencing trauma injuries will help measuring the in-vivo effect of TXA.

Figure 4.1: Exposure-response profile of the average observed clot lysis at 30 minutes after the maximum amplitude following four single doses of TXA in 6 healthy dogs



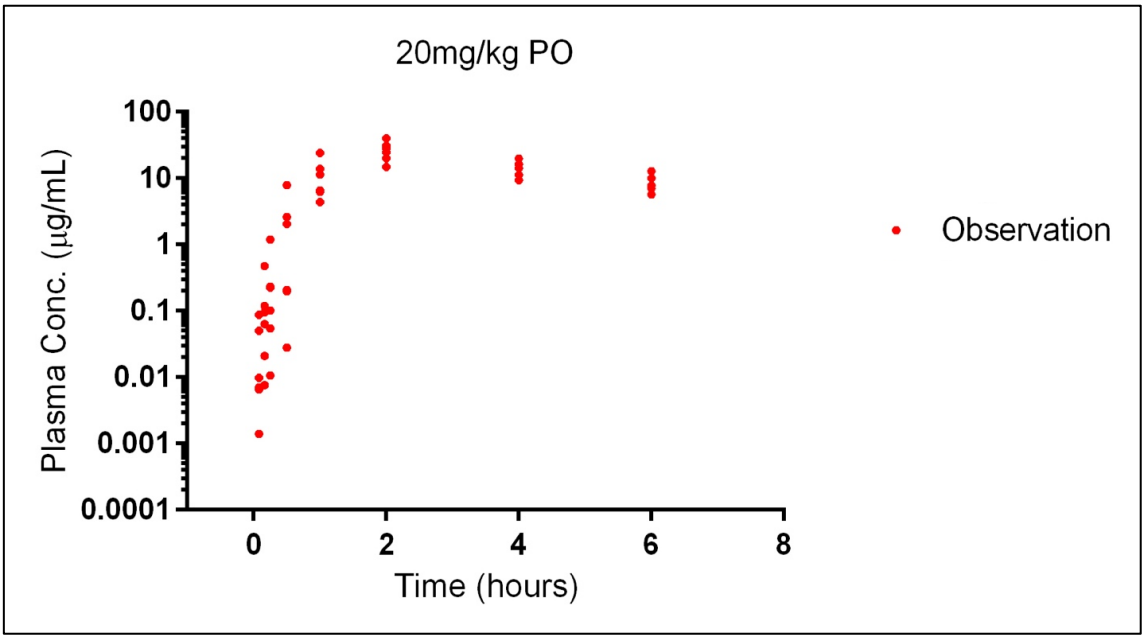
Y-axis denotes clot lysis percentage after 30 minutes from the maximum amplitude; X-axis denotes the time after TXA administration; red circles denote TXA10 mg/Kg intravenous 10 minutes infusion dose; red squares denote TXA 20 mg/Kg intravenous 10 minutes infusion; upward pointing triangles denote TXA 15 mg/Kg oral dose; downward pointing triangles denote TXA 20 mg/Kg oral dose; ⌶ denotes standard deviation from the mean of the averaged observed data.

Figure 4.2: Observed concentrations-time PK profile of TXA 15 mg/Kg oral dose in 6 healthy dogs (pooled data);



Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); red circles denote observed concentrations data.

Figure 4.3: Observed concentrations-time PK profile of TXA 20 mg/Kg oral dose in 6 healthy dogs (pooled data)



Y-axis denotes plasma drug concentrations in logarithmic scale (µg/mL); X-axis denotes time (hours); red circles denote observed concentrations data.

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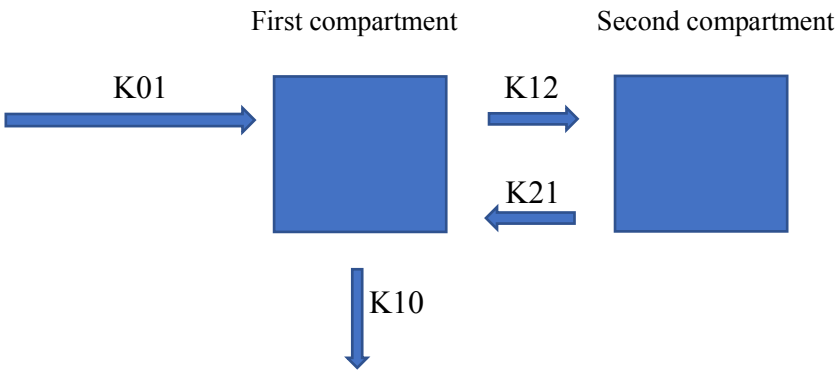
Appendixes

Appendix 1: Two-compartment pharmacokinetics model with first order elimination.



K01: Infusion rate or oral absorption
K12: Transfer rate to the second compartment
K21: Transfer rate to the first compartment
K10: Elimination rate

Appendix 2: Observed tranexamic acid (TXA) concentrations in plasma (μg/mL) of 4 single doses in 6 healthy dogs.

TXA concentrations (μg/mL) in plasma following 10mg/Kg IV 10 minutes infusion dose.

Time (minutes)	**5**	**10**	**15**	**30**	**60**	**120**	**240**	**360**
Dog #1	0.22	5.09	8.60	20.50	17.09	10.77	4.91	2.23
Dog #2	7.12	36.63	47.52	25.54	12.66	6.19	2.83	1.43
Dog #3	11.44	60.22	45.11	26.91	16.89	6.66	2.02	1.14
Dog #4	37.96	66.38	31.64	14.96	10.55	4.11	1.42	0.61
Dog #5	30.95	64.92	54.60	32.79	16.58	7.29	2.71	1.41
Dog #6	23.96	62.83	53.87	32.66	15.40	7.21	2.94	1.14

TXA concentrations (μg/mL) in plasma following 20mg/Kg IV 10 minutes infusion dose.

Time (minutes)	**5**	**10**	**15**	**30**	**60**	**120**	**240**	**360**
Dog #1	50.64	101.14	89.30	53.04	36.44	13.64	4.86	2.27
Dog #2	310.10	106.10	83.32	52.91	33.27	13.80	5.45	2.36
Dog #3	176.12	160.77	96.68	54.93	35.27	14.44	5.03	2.50
Dog #4	66.85	110.53	76.55	50.38	27.84	11.29	3.34	1.52
Dog #5	99.21	197.08	128.85	71.89	37.53	15.61	5.15	2.70
Dog #6	63.93	193.62	114.06	68.16	30.75	12.25	4.21	2.04

TXA concentrations (μg/mL) in plasma following 15mg/Kg PO dose.

Time (minutes)	**5**	**10**	**15**	**30**	**60**	**120**	**240**	**360**
Dog #1	0.00	0.01	0.01	0.04	2.11	10.80	9.16	6.11
Dog #2	0.00	0.00	0.00	0.00	0.02	1.16	6.48	6.72
Dog #3	0.00	0.00	0.00	0.05	5.93	13.33	11.36	5.90
Dog #4	0.00	0.00	0.02	0.64	7.91	17.77	9.97	4.36
Dog #5	0.00	0.01	0.02	1.12	5.41	10.19	8.52	5.65
Dog #6	0.02	0.02	0.03	0.04	2.86	16.18	15.48	8.73

TXA concentrations (μg/mL) in plasma following 20mg/Kg PO dose

Time (minutes)	**5**	**10**	**15**	**30**	**60**	**120**	**240**	**360**
Dog #1	0.00	0.06	0.23	2.05	13.85	14.81	9.37	7.05
Dog #2	0.01	0.02	0.05	0.20	6.27	20.10	14.27	7.84
Dog #3	0.01	0.01	0.01	0.03	6.55	40.21	19.81	10.03
Dog #4	0.05	0.47	1.18	7.85	24.21	27.89	11.33	5.69
Dog #5	0.01	0.12	0.22	2.62	11.36	31.19	16.04	10.08
Dog #6	0.09	0.10	0.10	0.21	4.39	24.63	16.28	12.79