Single Dose Pharmacokinetics of Pimobendan in Healthy Horses

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Academic Abstract

Few drugs are available to treat congestive heart failure and other cardiac diseases in horses. Pimobendan is an inodilator drug approved as Vetmedin® for treatment of canine cardiac disease. Previous research shows that pimobendan increases heart rate and contractility following intravenous administration in horses. The pharmacokinetics of oral pimobendan have not been investigated in horses. The hypothesis of this study was that pimobendan would be absorbed following oral administration to healthy adult horses and reach concentrations known to be therapeutic in other species. Additional objectives were to compare the absorption of compounded pimobendan capsules (C) and suspension (S) to Vetmedin® (V) and determine the effects of sample site on plasma drug concentrations in a pilot study using two horses. These two horses received C, S, or V (0.5 mg/kg via oral syringe, once) following a minimum 10 hour fast, using a crossover design with a minimum 1-week washout period. Samples were collected simultaneously from lateral thoracic and jugular catheters before and after drug administration at predetermined time points. Differences between formulation and sample site were analyzed by one-way ANOVA. After evaluation of the data from the initial 2 horses, an additional 4 horses received pimobendan, in the form of Vetmedin tablets® (V), in a similar manner. Only jugular samples were collected at the same predetermined time points. Plasma concentrations were determined by ultraperformance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) and pharmacokinetic parameters determined by noncompartmental analysis. No significant differences were noted between formulations or sample site ($P < 0.05$). Concentrations in compounded formulations were 88%(S) and 90%(C) of label. For V, mean

(±SD) maximum plasma concentration (C_{max}) was 4.96 ± 2.13 ng/mL at 2.17 ± 0.98 hours, and area under the curve (AUC0-∞) was 22.1 ± 8.8*ng/mL. Concentration of the active metabolite of pimobendan, o-desmethyl-pimobendan, was below the limit of detection (0.07ng/mL) for all samples. At 0.5mg/kg orally, pimobendan plasma concentrations were considerably lower than reported in dogs and other species. There was no evidence of oral transmucosal absorption. Pimobendan was poorly absorbed in horses, regardless of formulation, and appears unlikely to have clinical effects.

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General Audience Abstract

The objective of this study was to determine the pharmacokinetics (i.e. evaluation of the drug concentration in the bloodstream over time by means of mathematical modeling) of pimobendan in healthy horses. Pimobendan is a drug used to treat two types of heart disease, myxomatous mitral value disease and dilated cardiomyopathy, in dogs. These diseases often lead to a syndrome, congestive heart failure (CHF), that has high mortality. CHF in horses also has high mortality, and treatments for horses in CHF are based on information from other species. In this study, the FDA approved formulation of pimobendan, Vetmedin®, was administered to six healthy mature horses at a dose of 0.5 mg/kg, which is twice the amount typically administered to dogs in a single dose. Additionally, we gave two of the horses compounded (uniquely formulated) pimobendan capsules and suspension to evaluate their equivalence to Vetmedin® tablets. We serially collected blood samples to measure plasma concentrations of pimobendan after administration of each drug. Our results showed that all three formulations of pimobendan lead to similar blood concentrations in each of the two horses individually. No formulation of pimobendan resulted in plasma levels of pimobendan known to be effective in other species. Overall, the average plasma concentrations of pimobendan in these horses was very low, it was approximately $1/10^{th}$ the amount reported in canine pharmacokinetic studies of pimobendan. In conclusion, we determined that at a 0.5 mg/kg dose orally, pimobendan is poorly absorbed in horses and seems unlikely to have medical effects.

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Attributions

The execution of this research project and the writing of this manuscript was possible due to the contributions of the following individuals:

Harold McKenzie, DVM, MS, MSc (Vet Ed), DACVIM (LAIM) is a Professor of Large Animal Internal Medicine at the Virginia-Maryland College of Veterinary Medicine. Dr. McKenzie was integral in designing the study, securing funding, preparing for sample collection, collecting samples, and contributed to the preparation of the manuscript.

Jennifer Davis, DVM, MS, PhD, DACVIM (LAIM), DACVCP is a Professor of Clinical Pharmacology at the Virginia-Maryland College of Veterinary Medicine. Dr. Davis was integral in designing the study, performed sample analysis, data analysis and interpretation, and provided guidance for preparation of the pharmacokinetic manuscript.

McAlister Council-Troche was responsible for the assay development, and sample analysis, and preparation of the methods for the manuscript.

Katie Wilson, DVM, MS, DACVIM and Krista Estell, DVM, DACVIM contributed to study design and manuscript preparation

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Chapter 1: Introduction

The use of inotropic drugs for the management of acute and sometimes chronic congestive heart failure (CHF) is an integral part of managing this condition. Inotropes increase contractility of the myocardium, which increases cardiac output. Pimobendan is a drug with inotropic and vasodilatory properties that is used extensively in dogs, and occasionally other species, for the management of CHF. The FDA approved formulation of pimobendan, Vetmedin®, is labeled for the management of CHF in dogs with atrioventricular valvular insufficiency or dilated cardiomyopathy. Pimobendan has also been labeled for delaying the onset of congestive heart failure in dogs with Stage B2 preclinical myxomatous mitral valve disease (Vetmedin®-CA1). There are currently limited therapeutic options for managing CHF in horses. Therefore, there is a need for efficacious drugs for managing CHF in horses.

This thesis is compiled and formatted to discuss the use of pimobendan in human and veterinary medicine and the potential for use in equids specifically. The literature review will provide background information on cardiac physiology, the pathophysiology of CHF, and common causes of CHF in humans, dogs, and horses. Additionally, the use of inotropic and other drugs for management of CHF in these species and the pharmacology of pimobendan will be discussed. The principal portion of this document is a manuscript reporting the findings of a pharmacokinetic study investigating pimobendan in healthy horses. In closing, the directions of future research for use of pimobendan in horses will be addressed.

Chapter 2: Literature Review

Cardiac Physiology

Physiology of Cardiac Muscle

The pumping action of the heart is achieved via the coordinated contractility of the cardiac muscle. Cardiac muscle is unique in its structure, differing from both smooth muscle and skeletal muscle.[1] While cardiac muscle shares similarities to skeletal muscle, cardiac muscle faces an exceptional challenge—to continuously, rhythmically contract and relax for years and years on end. There are subtle but significant differences in the configuration of cardiac muscle compared to skeletal muscle.[2] Due to high energy demand, mitochondria occupy approximately 20-30% of the volume of cardiomyocytes, compared to 2-11% of the volume of skeletal myocytes.[3] The striation of cardiac muscle has a distinctive pattern (less organized compared to skeletal muscle) with shorter, single or binucleated cells that are connected via intercalated discs.[1,4] These intercalated discs contain gap junctions that propagate action potentials, allowing the impulse to travel rapidly so that the heart beats as a single unit, rather than the cardiomyocytes beating separately.[5] This coordinated propagation of action potentials from one cell to the next is known as electrical coupling, meaning that when one cardiomyocyte becomes electrically stimulated, an action potential quickly occurs in all of them.[6]

Like skeletal muscle, cardiac muscle is made up myofibrils, a portion of which is called a sarcomere. Sarcomeres are considered the functional units of muscle contraction.[7] Sarcomeres contain a central myosin-rich dark anisotropic (A) band and two actin-dominated light isotropic (I) bands.[8] Myosin (thick) and actin (thin) are filamentous proteins.[2] A myosin filament is made

up of many myosin molecules, and each molecule has a lobular projection known as the head.[9] This head is part of the cross-bridge unit that attaches to the actin filament during muscle contraction.[10]

Actin filaments are made up of F-actin molecules, tropomyosin molecules, and troponin molecules.[11] F-actin and tropomyosin molecules are longitudinally spiraled around each other.[12] The troponin molecules are made up of three subunits (troponin I, troponin T, and troponin C) and are dispersed intermittently along the tropomyosin strands.[13] Troponin I has a strong affinity for actin, troponin T has a strong affinity for tropomyosin, and troponin C has a strong affinity for calcium.[14] Troponin, with the different actions of its subunits, is responsible for anchoring the actin and myosin molecules together and for facilitating myofibril contraction.[12] When the sarcomere is at rest, the active binding sites on the actin molecule are inhibited or physically covered by the troponin-tropomyosin complex.[10] However, when calcium is present, calcium ions bind to the troponin complex, causing a conformational change that uncovers active binding sites on the actin molecule.[15] Myosin cross-bridges are then able to bind with these active sites on the actin molecule, which allows contraction of the myofibril to proceed.[9]

The cardiomyocytes' need for efficient movement of calcium cannot be understated. The plasma membrane of the cardiomyocyte is called the sarcolemma, and highly branched invaginations of this membrane are known as transverse tubules (t-tubules).[16] T-tubules regulate the resting membrane potential and action potential initiation and regulation.[17] T-tubules extend internally into myocytes and are rich in ion channels and signaling molecules that enable synchronous calcium release throughout the entire cell.[18] Perhaps the most important ion channels in the t-tubule are the voltage-gated L-type calcium channels (LTCCs).[17] In the t-

tubule, these channels are in close proximity to ryanodine receptor channels (RyRs), which are calcium sensing and release channels located in the sarcoplasmic reticulum.[1]

The sarcoplasmic reticulum is a specialized endoplasmic reticulum of muscle cells that stores calcium.[19] The sarcoplasmic reticulum has two pumps that regulate the amount of calcium present in the cytoplasm and thus available for diffusion along the myofibril—the RyRs and the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA).[20] SERCA pumps calcium back to the sarcoplasmic reticulum and away from the myofibrils.[21] This is important for relaxation of cardiac myofibrils, as the myofibril contraction will be sustained as long as the calcium concentration remains high.[22]

The mechanism by which an action potential causes the cardiac myofibrils to contract is called excitation-contraction coupling (ECC).[23] The t-tubules are the starting highway for the journey of ECC.[18] An action potential spreads along the membranes of the t-tubules reaching LTCCs, and these channels open to release calcium into the cell.[17] The increased concentration of calcium in the cytoplasm activates RyRs, which in turn releases stored calcium from the sarcoplasmic reticulum to the cytoplasm.[18] This calcium then binds to troponin to initiate cross-bridge formation and myofibril contraction.[12] The strength of cardiomyocyte contraction is dependent upon calcium ions from the t-tubules, as the sarcoplasmic reticulum is less well developed in cardiac muscle compared to skeletal muscle.[24] Additionally, the t-tubules in cardiac muscle have a diameter ten times greater than those in skeletal muscle, allowing for a greater volume of calcium in the t-tubule lumen.[17]

In contrast to skeletal muscle, cardiac muscle contraction is under involuntary control[25]. The cells of the myocardium can be divided into two types, those with a working myocardial phenotype and pacemaker cells.[26] “Worker” cells have an action potential that is prolonged

compared to nerve and skeletal muscle cells (hundreds of milliseconds vs. 3-5 milliseconds).[2,23] These cells have a stable resting membrane potential of -90 millivolts, and the action potential is marked by a plateau phase due to an opening of LTCCs and calcium entering the cell.[2] Pacemaker cells have an unstable resting membrane potential of -50 millivolts, and spontaneous depolarization occurs because of an ionic pacemaker current known as "funny" current.[2,27] Funny current is a mixed sodium/potassium inward current that is activated by hyperpolarization of the cell membrane; once activated these currents drive depolarization during diastole (relaxation of the heart muscle) which initiates a new action potential for electrical transduction in the heart.[27]

The cardiac cycle

The cardiac cycle refers to the events that occur in the heart during one heartbeat.[28] Each heartbeat is started by the spontaneous generation of an action potential by the pacemaker cells in the sinoatrial (SA) node.[26] The SA node is located in the right atrium, and once activated the action potential spreads rapidly through both atria before going to the atrioventricular (AV) node.[29] Conduction of the action potential through the AV node is slow mainly because of decreased numbers of gap junctions between cells; this allows the atria to contract before the ventricles.[5] Ventricular contraction (i.e. systole) is aided by the His-Purkinje system, which is excited after the slight delay of the action potential in the AV node.[30] The His-Purkinje system is the neuronal pathway in the ventricles; right and left bundle branches travel down the ventricular septum and terminate in a vast network of Purkinje fibers, which penetrate about one-third of the way into the cardiac muscle.[31]

The cardiac cycle begins with the SA node initiating contraction of the atria (arial systole).[32] Atrial systole occurs during ventricular diastole; the atrial contraction provides an

additional 20% of filling volume to the relaxed ventricle.[33] The AV valves (i.e. tricuspid and mitral valves) close, and the pressure in the ventricles rapidly rises as the action potential reaches the Purkinje fibers and the ventricular muscles contract, beginning the period known as isovolumic contraction.[34] This event is the start of systole. Once the pressure in the ventricles exceeds the pressures in the aorta and pulmonary artery, the semilunar valves (aortic and pulmonary valves) open, causing ejection of blood out of the ventricles for delivery to the lungs and systemic circulation.[32,35] Systole ends when the semilunar valves close and isovolumetric relaxation begins, a period when the ventricles quickly relax and drop the pressure in the ventricles substantially.[36] During diastole, the AV valves open and blood fills the ventricles, starting the cycle over again.[35]

The contraction of the cardiac muscle is affected by two forces, preload and afterload.[34] Preload represents the factors that contribute to ventricular wall tension at the end of diastole.[37] Afterload is the factors that contribute to total myocardial wall tension during systolic ejection, which is largely, but not only, the pressure in the aorta.[34,37] By most definitions, preload is considered equivocal to end-diastolic pressure in the left ventricle.[34,38]

Regulation of cardiac function

To meet various physiologic and pathological challenges, there is a need for instantaneous changes in the rate and strength of the heart's contractions.[39] These changes are regulated by the autonomic nervous system, which is subdivided into the sympathetic nervous system and the parasympathetic nervous system.[40] There are both sympathetic and parasympathetic nerve fibers that directly innervate the heart, the main parasympathetic fibers being the vagus nerves.[34,41] Parasympathetic stimulation causes a marked decrease in heart rate and a minor decrease in cardiac muscle contractility.[42] The vagal fibers mainly terminate in the

atria and to a lesser degree in the ventricles, highlighting the purpose of parasympathetic stimulation for decreasing heart rate rather than decreasing the strength of ventricular contraction.[34] Sympathetic stimulation can drastically increase heart rate and can also double the force of cardiac muscle contraction.[40,43]

Baroreceptors are nerve endings located in the walls of arteries that are stimulated when stretched.[44] The aortic arch has a vast number of baroreceptors, and signals from these baroreceptors are transmitted through the vagus nerves to the brain.[45] This excites the vagal parasympathetic center in the medulla, which in turn signals for a decrease in heart rate, cardiac contractility, and vasodilation throughout the peripheral circulatory system.[46] Collectively, this decreases afterload and preload forces on the heart.[47] The opposite affects will occur when the baroreceptors sense a decrease in aortic wall tension, thereby reflexively increasing the pressure in the peripheral vasculature.[48]

Cardiac output: physiology and regulation

The volume of blood ejected from the left ventricle during each contraction is known as stroke volume, which is measured in liters.[39,49] The effectiveness of the heart to send blood to the rest of the body is also modulated by heart rate.[50] A decrease in stroke volume can be compensated for by an increased heart rate, thereby keeping the volume of blood pumped to the body per minute the same.[51] This concept summarizes cardiac output. Cardiac output is defined as the liters of blood pumped from the heart per minute, i.e. stroke volume multiplied by heart rate.[39,49] Since cardiac output represents the volume of blood distributed to all the tissues in the body, it is perhaps the most important factor to consider when examining the functional capacity of the cardiovascular system.[52] For the normal heart, cardiac output is maintained in proportion to overall metabolic activity.[50]

Both peripheral and cardiac mechanisms affect the regulation of cardiac output. Perhaps the most significant cardiac mechanism for this regulation is the Frank-Starling relationship.[52] This mechanism shows that muscle force and stroke volume are proportional to sarcomere length and preload, respectively.[53] This means that the heart possesses an intrinsic ability to adapt to increasing volumes of blood (i.e. increased venous return), because the more the cardiac sarcomeres are stretched, the greater the force of contraction.[52] Therefore, in a normal healthy heart, with increased preload there is an increase in stroke volume, and therefore an increase in cardiac output.[2]

Other cardiac mechanisms of regulating cardiac output occur because of stretching of the right atrium. Stretching of the SA node causes increased rhythmicity of the pacemaker cells, thereby increasing heart rate.[54] Stretching of the atrium also initiates the Bainbridge reflex, which is a vagal nervous reflex stimulated by an increase in blood volume.[55] The signal travels via the vagus nerves to the medulla of the brain, and subsequently the signal is transmitted back through vagal and sympathetic nerves to increase the heart rate and cardiac contractility.[56]

The venous return to the heart is determined by the dynamics occurring in the peripheral circulation.[52] Smooth muscle tone in vessels is regulated by the sympathetic nervous system and circulating vasoactive hormones such as angiotensin II.[57] The force exerted by the vessels on the circulating blood stream is called systemic vascular resistance (SVR).[58] Increased SVR, a significant contributor to afterload, will cause a reduction in cardiac output; while conversely, a decrease in SVR will lead to increased cardiac output.[59]

Congestive Heart Failure

Congestive heart failure (CHF) generally carries a poor long-term prognosis, regardless of species.[60-64] It is a condition that still causes significant morbidity, despite remarkable advances in heart failure medicine over the last few decades.[65-67] CHF is not a specific disease, but rather a clinical syndrome.[68,69] "Congestive" refers to the formation of edema and/or venous distention, which accumulates either subcutaneously, intraabdominally (as in the case of right-sided heart failure), in the pulmonary space (as in the case of left-sided heart failure), or in multiple locations (as in the case of biventricular heart failure). The clinical signs of "left-sided" and "right-sided" heart failure frequently overlap.[49] Therefore, evidence of one or more areas of congestion is not necessarily pathognomonic for which side of the heart is affected. Heart failure has a plethora of etiologies, many of which are beyond the scope of this review. Irrespective of the cause, the goals of managing CHF include improving/stabilizing hemodynamic function and relieving systemic and pulmonary congestion.[70]

Pathophysiology of CHF

Regardless which area of the heart is dysfunctional, the end result of heart failure is decreased cardiac output.[71] Adequate cardiac output is essential for perfusion and function of the body's tissues and organs. Physiological feedback mechanisms exist to correct a decrease in cardiac output. Unless the primary cardiac functional issue is corrected, these initially beneficial compensatory mechanisms will persist and lead to life-threatening consequences.[72] A decrease in cardiac output is detected by baroreceptors in the left ventricle, aortic arch and carotid sinus. These baroreceptors, along with renal feedback mechanisms, initiate signaling for compensatory

neurohormonal systems that eventually become maladaptive—most notably the sympathoadrenergic system and the renin-angiotensin-aldosterone-system (RAAS).[73]

It has been well established that sympathetic nervous system (SNS) over stimulation is present in human and small animal patients in heart failure.[49,72,74] This has been evidenced by increased concentrations of plasma norepinephrine (NE) in CHF subjects compared to healthy controls.[75,76] Additionally, increased plasma levels of NE have been correlated with increased mortality in human CHF patients.[76] Plasma levels of NE in the setting of CHF has not been investigated in horses. While SNS stimulation is initially beneficial for increasing and maintaining cardiac output, chronic stimulation leads to deleterious effects. These effects include increased afterload via peripheral vasoconstriction, persistent tachycardia, and amplified myocardiocyte automaticity that can lead to arrhythmias.[49,77] Additionally, sympathetic stimulation of renal nerves leads to reduced renal perfusion and RAAS activation.[78]

While it is generally thought that SNS activation occurs before RAAS activation, it is also theorized that SNS over stimulation occurs secondary to RAAS activation, because angiotensin II has a stimulatory effect on norepinephrine release from sympathetic nerve terminals and adrenergic receptor responsiveness.[49,72,79] Regardless of which event comes first, RAAS is initiated by release of renin from the juxtaglomerular cells in the macula densa of the distal tubule occurs secondary to decreased blood pressure and sodium delivery.[80] Renin converts the plasma protein angiotensinogen to angiotensin I (AngI), and then the pulmonary capillaries release angiotensin-converting enzyme (ACE) to convert AngI to angiotensin II (AngII).[77] Ang II then stimulates release of aldosterone from the adrenal cortex.[49,73] Aldosterone binds to mineralocorticoid receptors in the distal tubule and causes sodium retention and potassium excretion.[81] AngII can also act directly on the proximal tubule of the nephron to promote sodium

and water retention. Additionally, AngII is a potent vasoconstrictor.[49,77] Altogether, this increases extracellular fluid volume and blood pressure, hydrostatic pressures overwhelm the draining capabilities of the lymphatic system, and tissue edema develops.[82] This increase in extracellular fluid volume and blood pressure will also exacerbate the development of pulmonary edema or ascites (increased fluid in the abdomen), dependent upon where the primary cardiac lesion is located and the abilities of the lymphatic system to compensate.[49]

In line with the principles of homeostasis, neurohormonal mechanisms exist to counteract the fluid retention caused by overzealous RAAS activation. With mechanical stress of the atrial wall, atrial natriuretic peptide (ANP), and to a lesser degree brain natriuretic peptide (BNP), is released to trigger vasodilation, diuresis, and natriuresis.[83] As such, concentrations of ANP, BNP, and their N-terminal fragments in the blood increase in the setting of CHF.[49,77] Unfortunately with chronic activation of RAAS, the actions of the natriuretic peptides become obtunded, and therefore clinical signs of CHF develop.[77]

Causes of CHF and categorization of heart failure in humans

In humans, the leading cause of heart failure is myocardial dysfunction.[84] Myocardial dysfunction occurs from one or more primary disease processes. In developed countries, these primary diseases are most commonly coronary artery disease (CAD) and hypertension.[85] CAD, sometimes referred to as ischemic heart disease (IHD), can lead to an acute myocardial infarction.[86] The increase in afterload from hypertension causes myocardial dysfunction via myocyte hypertrophy.[87] A recent global, prospective observational study on CHF found that hypertensive heart failure was the most frequent etiology in women, while ischemic heart failure was the most frequent etiology in men.[88] The broad category of cardiomyopathies (hypertrophic, dilated, restrictive, arrhythmogenic right ventricular, and unclassified cardiomyopathies[89])

follows these two diseases in prevalence as a cause of myocardial dysfunction.[90] Pathology of the valves, endocardium, pericardium, or arrhythmias are less common causes of heart failure compared to myocardial dysfunction.[66,88] A cause of heart failure unique to humans is rheumatic carditis, a serious manifestation of rheumatic fever.[91] Group A streptococci, which are only pathogenic to humans, are responsible for triggering the immune-mediated inflammation in rheumatic carditis.[92]

Heart failure in humans is divided into phenotypic categories to aid in prognostication and treatment recommendations.[63,85] These categories are created from measuring the left ventricular ejection fraction (LVEF), whereas heart failure with preserved ejection fraction (HFpEF) is differentiated from heart failure with mildly reduced ejection fraction (HFmrEF) and heart failure with reduced ejection fraction (HFrEF).[69] The cut-offs for these categories are LVEF ≥ 50%, LVEF 41-49%, and LVEF ≤ 40%, respectively.[70,85]

Causes of CHF in dogs

Myxomatous mitral value disease (MMVD) is the most common etiology leading to CHF in dogs.[93,94] The pathogenesis of this disease is not completely understood, although an inherited component is evident in some breeds (e.g. Cavalier King Charles Spaniels (CKCS), Dachshunds).[95,96] The term "myxomatous" refers to the histopathologic changes that occur in the valve leaflets and chordae tendineae. The disorganization of collagen fibrils, changes in proteoglycan content, and activated myofibroblasts lead to deformation of the mitral valve and subsequent regurgitation.[93,97,98] This disease has a chronic progression; therefore, except for dogs with a genetic predisposition (CKCS), it is most frequently detected in middle-age to older dogs.[99,100]

Following MMVD, dilated cardiomyopathy (DCM) is the next most prevalent cause of cardiac disease in dogs.[101,102] DCM is the most common cause of heart failure in large breed dogs, particularly Doberman Pinchers and Boxers.[102,103] A plethora of other conditions can lead to CHF in dogs, including but not limited to diseases of the pericardium, endocardium, tricuspid or semilunar values, and rhythm disturbances.[104]

Causes of CHF in horses

Horses are often used as athletes in some capacity, and a healthy cardiovascular system is of utmost importance for their athletic potential.[105] Furthermore, because they are carrying riders or pulling drivers in a cart the health of the cardiovascular system gains extra importance, as sudden collapse from a cardiac event is a known risk factor for equestrian injury.[106] Heart failure substantially increases the risk of sudden collapse in horses, and therefore a horse with signs of CHF should not be ridden or exercised.[107]

CHF is not a common condition in horses and has been reported to make up less than 1% of equine referral hospital cases, and only 10% of cases presented for cardiac work-up specifically. The mean age of CHF cases in three retrospective studies were 7.6 years, 8.5 years and 13.0 years, with the latter study excluding horses <2 years of age.[60,108,109] No breed predilection has been identified. One study found that Warmbloods were overrepresented (27/28 cases) in their CHF cohort but Warmblood horses made up almost 70% of the hospital's caseload.[108] The following is not an exhaustive list of all the causes of CHF reported in horses. This list includes causes of equine heart failure that can become chronic in nature and have the potential to improve with medical therapy.

Valvular insufficiency: Two studies have shown that mitral valve regurgitation was the most common valvular disease associated with CHF in horses.[60,108] Aortic valve regurgitation is

quite common in older horses, but in most horses the regurgitation remains mild and progression to CHF is not common.[108,110,111] Isolated tricuspid or pulmonic valve regurgitation seems to rarely cause CHF unless a rupture of the chordae tendinea of the tricuspid valve or tear of the pulmonary valve occurs.[112,113] Valvular endocarditis (VE) can cause signs of CHF, as reported in 35% of VE cases at one institution.[114]

Cardiomyopathies: Myocardial damage in horses is most often subclinical. However, severe acute or chronic myocarditis can progress to overt heart failure.[112,115] Dilated cardiomyopathies are rare in horses and are classified as idiopathic or occur secondary to toxic insults or sustained ventricular tachycardia.[116] While not common, one of the best described causes of toxic myocarditis is ionophore toxicity. Horses are extremely sensitive to ionophores as compared to other species, likely due to a difference in the horse cytochrome P450 metabolism of ionophores.[112,117] Often, cases of ionophore toxicity will die acutely, and histopathologic evidence of cardiac injury cannot be found on necropsy.[118] However, some surviving horses will develop a long-term cardiomyopathy and later present with signs of CHF.[119]

Pericarditis: For inflammation of the pericardium to lead to CHF, compression must occur that exceeds the right ventricular filling pressure, thereby decreasing diastolic function.[120] This compression is commonly known as “cardiac tamponade” and leads to impaired systolic function and right-sided CHF.[116,120] The detection of clinical signs of right-sided CHF was significantly associated with severe pericardial effusion in one study.[121] This same study demonstrated successful treatment of pericarditis (14/14 cases) with antimicrobials, +/- corticosteroids, and/or pericardial drainage and lavage as indicated based on the suspected etiology of the pericarditis. Another more recent study showed that 24/33 (72%) thoroughbreds treated for pericarditis at a referral hospital survived to discharge.[122] The exception for a good

prognosis with regards to CHF secondary to pericarditis/pericardial effusion would be a suspicion of neoplasia.[123]

In conclusion, the overall prognosis for horses in CHF is grave, with most horses surviving less than 1 year.[60,68] Euthanasia is often recommended and chosen in the face of such a grave prognosis. The exception to this would be CHF due to effusive, idiopathic or infectious pericarditis, as these cases typically respond well to aggressive treatment.[121] However, treatment options for CHF with short-term efficacy exist, and may prolong the life of a horse in heart failure for a substantial amount of time.

Comparative aspects of the causes of CHF in humans, dogs, and horses.

In both dogs and humans, CHF is more often diagnosed secondary to acquired heart disease than congenital heart disease.[85,104,124] The most common cause of heart failure in people, myocardial dysfunction or ischemia secondary to CAD, is incredibly rare in dogs.[125] Nonetheless, canine models are often used to study CHF in human medicine.[126-128] The two most common causes of CHF in dogs, MMVD and DCM, have parallels in human medicine. Similarities exist with the pathology of the mitral valve in MMVD in both humans and dogs.[98,129,130] DCM is often an inherited condition in humans, and inheritance patterns are also evident in dogs.[102,131] MMVD and DCM in humans and dogs is an area where the causes of CHF in these species overlap, which provides opportunities for translational research. As in dogs, it is reported that mitral valve regurgitation is the most common underlying cause of congestive heart failure in horses.[108] Furthermore, it has been demonstrated that the affected valves of horses with gross cardiac abnormalities had myxomatous changes similar to dogs and humans.[132]

Diagnosing CHF in humans, dogs, and horses

Clinical history and exam findings

For all three of the aforementioned species, a diagnosis of CHF begins with a thorough clinical history and physical exam.[49,133] Clinical history items possibly supportive of CHF in all these species include exercise intolerance (e.g. shortness of breath with mild exercise), difficulty breathing at rest, and/or anorexia.[49,134] A human patient may report feeling fatigued, and likewise, dog and horse owners might report that their animal is less active or acting dull.[68,135,136]

Some of the physical exam findings for human CHF patients include jugular vein distension, dyspnea (shortness of breath), leg edema, abdominal distention, cachexia, and uniquely in humans—bendopnea (shortness of breath when bending over).[70,135,137] Cardiac cachexia is a type of muscle wasting attributed to proinflammatory cytokines, particularly tumor-necrosis factor-α, released secondary to heart failure.[49] The pro-inflammatory pathogenesis is similar to the muscle wasting seen in other chronic disease like cancer.[49,138] Dogs in CHF failure will often present with dyspnea or tachypnea (increased respiratory rate) and have a heart murmur.[136] Dogs can also present with jugular vein distension, pulmonary crackles on auscultation, ascites, cachexia, and/or subcutaneous edema.[49,136] Coughing is a clinical sign suggested to be prevalent in dogs with CHF, but this has not been found to be the case in humans with CHF.[139] Common physical exam findings for a horse in CHF are the same as those listed above for dogs.[68] Ventral body wall and distal limb edema is a more common manifestation of CHF in horses than in dogs.[49] A dramatic sign of left-sided heart failure in horses is frothy nasal discharge, which is a testament to either the severity or the acuteness of the heart failure.[112] Abnormalities on cardiac auscultation of a horse in CHF may include an attenuated first heart

sound due to decreased contractility or effusion, a loud cardiac murmur (grade 3/6 or greater), an arrhythmia (most often atrial fibrillation), or all of the above.[68]

Imaging

Thoracic radiographs can be used in humans, dogs, and horses to look for evidence of cardiomegaly or pulmonary edema.[68,70,140] While useful as a cursory diagnostic to also investigate for evidence of non-cardiogenic pulmonary disease, thoracic radiographs have been shown to be less sensitive than thoracic ultrasound in humans in identifying pulmonary edema.[141] Diagnostic quality thoracic radiographs are challenging to acquire in adult horses due to their size, and it is not always possible to view the entire heart in one image.[142] For this and many other reasons, such as the ability to observe the heart beating in real time, echocardiography (EC) is the standard imaging modality used across species to evaluate cardiac function when CHF is suspected.[143-145] Cardiac structure, function, and abnormalities of the myocardium, heart valves, and pericardium can be visualized via transthoracic EC.[135,146]

The functional echocardiographic variable used extensively in human heart failure assessment is ejection fraction of the left ventricle (LVEF).[147] Ejection fraction is calculated by dividing stroke volume (which is calculated by subtracting the end-systolic volume from the end-diastolic volume (EDV) measured on EC) by EDV and then multiplying by 100 to obtain a percentage.[148] LVEF can be obtained on echocardiography of the dog and horse.[146] However, preservation of LVEF varies based on disease etiology, and no veterinary study has examined LVEF as a main variable in treatment or outcome in CHF.[49]

Echocardiography is very often successful in identifying the cause of CHF pre-mortem in horses.[60,109] Rounded, enlarged cardiac chambers are a common echocardiographic finding in cases of CHF secondary to mitral valve insufficiency. The end-diastolic left ventricular diameter

will often be greater than 16.0 cm.[112] In the left parasternal long-axis view, a left atrial measurement of >13.5 cm in a mature horse is considered enlarged.[109] Normally, the pulmonary artery diameter should be less than the aortic diameter. Aortic dilation can occur in cases of chronic, severe aortic regurgitation, as well as with anatomic malformations (e.g. congenital defects, aneurysms). Conversely, the aortic diameter may be decreased with low cardiac output or systemic hypotension.[149] Hypomotility and reduced fractional shortening are general indicators of myocardial dysfunction often seen in CHF in horses.[112]

Biomarkers

Because concentrations of brain natriuretic peptide (BNP) and its N-terminal fragment (NT-proBNP) increase in the setting of CHF, these peptides have been used as biomarkers for heart failure in humans and dogs.[77,150,151] In humans and dogs, low circulating NT-proBNP concentrations have a high negative predictive value, making it a valuable rule-out test for CHF.[152] In humans with heart failure, higher levels of BNP and NT-proBNP are correlated with increased risk for adverse short-term and long-term outcomes, including cardiovascular death.[70] Atrial natriuretic peptide (ANP) has been investigated as a biomarker in horses, with conflicting results on its utility for detecting left atrial enlargement in horses.[153-156]

Treatment of CHF

Pharmacologic therapies for CHF in humans

Most patients in human pharmacologic clinical trials are selected based on their ejection fraction (EF) and subsequent heart failure (HF) classification.[70] For human patients with pre-clinical heart failure (Stage B) and LVEF ≤40%, angiotensin-converting enzyme inhibitors

(ACEi) are recommended to prevent CHF and reduce mortality.[157-159] Angiotensin II receptor blockers (ARB) can be used if the patient is intolerant of an ACEi (side-effects of ACEi include cough and angioedema).[160,161] Additionally, beta-blockers are recommended for Stage B HFrEF patients with CAD or myocardial ischemia.[70] Beta-blockers antagonize catecholamines, thereby reducing the deleterious effects of chronic sympathetic activation such as myocardial oxygen consumption and increased heart rate.[162,163]

For human patients with CHF (Stage C or D) standard treatments include a ACEi plus a beta-blocker, in conjunction with some combination of a mineralocorticoid receptor antagonist (MRA), sodium glucose cotransporter 2 inhibitor (SGLT2i), angiotensin receptor/neprilysin inhibitor (ARNi), and/or a diuretic as needed.[70] While MRAs show consistent improvements for HFrEF patients relating to all-cause mortality and HF hospitalizations, careful monitoring of the patient's potassium, renal function, and diuretic dosing is required.[164,165] ARNis are a combination of an ARB and an inhibitor of neprilysin, an enzyme that degrades natriuretic peptides and other vasoactive peptides.[166] SGLT2i drugs, which were classically prescribed for the treatment of type II diabetes, have been shown to reduce hospitalization for HF and cardiovascular mortality, regardless of whether diabetes was a co-morbidity or not.[160] Commonly used diuretics in human CHF patients are loop-diuretics such as furosemide and torsemide.[167] If the symptoms of CHF do not respond to moderate to high doses of a loop diuretic, a thiazide diuretic (e.g. metolazone, hydrochlorothiazide) can be used, but the patient needs to be monitored for the development of electrolyte abnormalities.[70]

Pharmacologic therapies for CHF in dogs

Pharmacologic therapies for dogs in CHF are more often studied in the context of the primary heart failure etiology, with large clinical trials including dogs diagnosed with MMVD,

DCM, or both.[168-170] Pimobendan in the only drug recommended for delaying the onset of signs of CHF in dogs with MMVD or DCM.[93,171] Its clinical use is discussed extensively in one of the sections to follow. Similar to studies in humans, ACEi drugs have been shown to improve survival in dogs with CHF.[170,172] Both furosemide and torsemide have good oral bioavailability in dogs, and these drugs demonstrated similar treatment success in dogs with CHF secondary to MMVD[173]. Spironolactone, a MRA drug, has been shown to be beneficial in dogs with clinical MMVD when used in combination with an ACEi and furosemide.[174,175]

Pharmacologic therapies for CHF in horses

Treatment options for CHF in horses are largely extrapolated from other species. Reports of horses medically treated for signs of CHF in the literature are sparse, and therefore the true efficacy of the various treatments tried is unknown.[176,177] Assuming that the physiology of CHF in horses is nearly identical to that in other species, the goals (reducing cardiac work by decreasing preload and afterload) and drugs available (for extra-label use) are largely the same.

A mainstay in the treatment of CHF in all species is reducing preload via diuresis. Furosemide is widely used in horses for diuresis and is recommended in the treatment of CHF.[178] Unfortunately, furosemide has poor oral bioavailability in horses and must be administered intravenously (IV) or intramuscularly (IM).[179] The diuretic effect of IV furosemide is rapid with a peak effect occurring within 15-30 minutes. The diuretic effect of IM furosemide is greater than IV furosemide due to a longer elimination half-life, which may be advantageous in the CHF case.[180] The pharmacokinetics and pharmacodynamics of torsemide, another loop diuretic that can be administered orally, have also been investigated in horses. While torsemide produced significant diuresis, the study horses also developed moderate pre-renal azotemia and electrolyte disturbances.[181] Recent evidence suggests that ACE inhibitors could be beneficial in horses with

mitral valve disease.[93,182] The pharmacokinetics and bioavailability of several ACE inhibitors have been investigated in horses, with benazepril demonstrating the best oral bioavailability.[183,184] Digoxin, which is discussed below, is a cardiac glycoside commonly used in horses with CHF—the pharmacokinetics of digoxin has been studied in healthy horses and horses with CHF.[185-187]

Inotropic drugs used in humans, dogs, and horses

The term *inotropy* is nearly synonymous with contractility of the heart. Inotropic agents improve the force of ventricular myocardium contraction, thereby increasing cardiac output.[188] All inotropes that have been used in clinical medicine to date cause one or both of the following events in the cardiac myocyte; increased concentrations of intracellular calcium or increased sensitivity of myofilaments to calcium.[189,190]

Digoxin, one of the oldest cardiovascular drugs still in use today, has a unique mechanism of action through inhibition of sodium-potassium ATPase.[191] This inhibition reduces the activity of the sodium-calcium exchanger, raising intracellular calcium concentrations in the cardiomyocyte.[192] A significant disadvantage to digoxin therapy is the need to monitor blood digoxin levels, as digoxin levels ≥ 1.2 ng/mL may be harmful and digoxin toxicity is a prevalent cause of hospitalizations secondary to adverse drug events in human patients.[192,193]

Dopamine and dobutamine, two sympathomimetic drugs, were developed after digoxin as inotropic therapies.[192] As β-agonists, they activate G-protein-linked receptors in the plasma membrane of the cardiomyocyte, which causes a cascade of events including the production of cyclic adenosine monophosphate (cAMP).[189] cAMP activates protein kinase A (PKA), which mediates processes that increase intracellular calcium, which through several mechanisms

increases the strength of myocardial contractility.[194] These β-agonists, however, have additional effects on the cardiovascular system that can be negative in context of heart failure, such as vasoconstriction (dopamine) or vasodilation (dobutamine).[192] In addition, rapid clearance from the body means that these drugs are ineffective unless administered as a constant rate infusion, and down regulation of β-receptors renders these drugs ineffective over time; in humans, dobutamine tolerance is noted in infusions lasting longer than 72 hours.[192]

Phosphodiesterase 3 inhibitors (PDE-3) prevent the breakdown of cAMP, which increases intracellular calcium levels in the cardiomyocyte, but decreases the amount of calcium available to vascular smooth muscle cells.[189,195] Therefore, PDE-3 inhibitors are also effective vasodilators. PDE-3 inhibitors such as amrinone, milrinone, and pimobendan all showed promise in initial human clinical trials as improvement in hemodynamic and quality-of-life variables were seen.[196-198] Calcium sensitizing drugs such as levosimendan and pimobendan increase the affinity of calcium to cardiac troponin C, which increases the force generated by cardiac myofibril unit.[190,199] Both drugs also inhibit PDE-3 (pimobendan more so than levosimendan) adding to their efficacy.[192,200]

Inotropic therapy is heart failure is controversial[190,201,202] as multiple clinical trials have found that inotropes failed to improve outcomes in heart failure patients, and in many trials were associated with significant increases in mortality.[189,203-205] While increasing the force of myocardial contractility intuitively seems beneficial, inotropes may also increase myocardial oxygen demand in the failing heart, which is already "energy starved" from excitation-contracting coupling dysfunction.[206,207] This is particularly important in the context of CAD and myocardial hypertrophy secondary to hypertension, where there is a decreased supply of oxygen and/or increased oxygen demand to the myocardium.[208,209] Therefore, the general consensus in

human heart failure medicine today is that inotropes should be used for palliative care of patients or for patients that are unresponsive to management with other medications.[85,190]

Today, only one non-sympathomimetic inotropic drug (milrinone) is labeled for treatment of acute congestive heart failure in the United States.[202,210] However, this drug is frequently used chronically, off-label, for patients with end-stage heart failure.[190] Digoxin in humans and pimobendan in dogs are the only inotropic drugs labeled for treatment of chronic heart failure in their respective species. In veterinary medicine, the use of pimobendan is considered standard of care for dogs with MMVD or DCM that are exhibiting signs of CHF and has recently been labeled for delaying onset of CHF. [93,169,211] Digoxin has been used extra-label in dogs for select cases of atrial fibrillation or DCM.[212,213]

Digoxin is often used in equine CHF cases for its positive inotropic effect.[185] Digoxin also has negative chronotropic effects, which makes it particularly indicated in horses in CHF with concurrent tachycardia and atrial fibrillation.[112] Due to a narrow therapeutic index, plasma levels of digoxin should be monitored in horses undergoing treatment. Clinical signs of toxicity include depression, anorexia, and cardiac arrhythmias, and typically occur when plasma concentrations exceed 2 ng/mL.[178]

Pimobendan

Drug properties and mechanism of action

The first generation of selective phosphodiesterase inhibitors, including pimobendan, were developed through experimental pharmacology in the 1980-1990s.[214,215] The inotropic agents widely available at the time, catecholamines and digoxin, had undesirable side-effects or resulted in pharmacologic tolerance over time.[192,216] Pimobendan surfaced as a unique PDE-3

inhibitor as it was also found to cause increased calcium sensitization of cardiac troponin C, the calcium binding subunit of the troponin-tropomyosin complex.[199,217]

The inotropic effects of pimobendan are the result of two mechanisms, calcium sensitization and PDE-3 inhibition.[218] PDE-3 enzymes remove cAMP in cardiac myocytes.[219] When present, cAMP activates PKA, and PKA phosphorylates three different substrates (phospholamban, ryanodine receptors, and L-type calcium channels) which leads to an influx of cytosolic calcium and calcium sequestration in the sarcoplasmic reticulum.[199,220] Inhibition of PDE-3 by pimobendan has the greatest positive inotropic effect in a healthy heart, whereas it has been suggested that calcium sensitization is the predominant inotropic mechanism for pimobendan in the failing heart.[221,222] Two studies, one in vitro and one in vivo, showed that pimobendan does not increase myocardial oxygen consumption.[215,223]

Pimobendan is oxidatively demethylated to its active metabolite, UD-CG 212 CL, also known as o-desmethyl-pimobendan (ODMP), in the liver.[224] ODMP is a more potent PDE-3 inhibitor compared to pimobendan, however, it is undetermined whether ODMP has calcium sensitizing properties or not.[222,225] ODMP may also have inotropic effects via antagonism of A_1-adenosine receptors; these receptors are a less recognized contributor to cAMP production and subsequent inotropy.[226] Pimobendan and ODMP are > 90% plasma protein bound, and ODMP is largely secreted in feces after conjugation with sulfate or glucuronic acid.[227]

Clinical use in humans

The initial clinical studies for pimobendan—performed on small groups of patients with heart failure—showed exciting results such as diminished preload and afterload[223], increased exercise tolerance[203], and decreased concentrations of plasma catecholamines.[228] A clinical trial comparing pimobendan to enalapril in patients with mild to moderate heart failure found that

both drugs improved exercise capacity and there was no difference in mortality between the two drugs.[229] During the 1990s, large clinical trials investigating the outcomes of patients treated with other inotropic drugs such as xamoterol, milrinone, and dobutamine began publishing results on increased risk of mortality for the treatment groups.[205,230,231] The first large clinical trial investigating pimobendan compared to a placebo was performed in Europe and named the Pimobendan in Congestive Heart Failure (PICO) trial.[203] Pimobendan did improve exercise duration, but no effects on peak oxygen uptake or quality of life scores were seen. Concerningly, pimobendan treatment had a hazard of death 1.8 (95% confidence interval 0.9 to 3.5) times higher than in the placebo group. This study, combined with the collective results from other clinical trials on inotropic drugs, lead to the discontinuation of pimobendan's clinical development in most countries.[194]

Japan has continued using pimobendan for the treatment of chronic heart failure. One Japanese study showed that after 52 weeks of treatment with pimobendan, chronic heart failure patients had improved physical activity scores and no difference in mortality rates compared to the placebo group.[232] In Japan, pimobendan is used in a subset of patients to improve physical activity.[233] One study showed decreased rates of hospitalization for five severe, chronic heart failure patients receiving pimobendan in conjunction with ACE-inhibitors, beta-blockers, and diuretics.[234]

Clinical use in veterinary species

Vetmedin® is a Food and Drug Administration (FDA) approved formulation of pimobendan that is labeled for the management of CHF in dogs with atrioventricular valvular insufficiency or dilated cardiomyopathy.[227] The evidence that pimobendan benefits dogs with clinical signs of CHF secondary to MMVD is substantial. Several studies have shown that

pimobendan in superior to ACE-inhibitors (ramipril, benazepril) in prolonging time to death or other adverse heart failure outcome.[168,235,236] Since pimobendan has been found to improve short-term cardiac function and long-term survival for dogs with clinical MMVD, it is a mainstay of therapy for dogs in CHF secondary to this condition.[93,237] Pimobendan also increases survival time for Doberman Pinchers with CHF secondary to DCM.[169,238]

Recently, an expert panel of veterinary cardiologists has recommended the use of pimobendan in dogs with MMVD even before clinical signs of congestive heart failure develop if the dog has echocardiographic and/or radiographic evidence of cardiac remodeling (i.e stage B2).[93] This recommendation comes from a landmark clinical trial, funded by Boehringer Ingelheim Animal Health (the producer of Vetmedin®), called the "Evaluation of Pimobendan In dogs with Cardiomegaly caused by preclinical mitral valve disease" (EPIC) trial. This study, which was prospective, blinded, randomized, and placebo-controlled, enrolled dogs that were ≥ 6 years of age, had no clinical signs of MMVD, but had evidence of MMVD on echocardiogram and had radiographic evidence of cardiomegaly. The primary endpoints of the study were development of left-side CHF, euthanasia for a cardiac reason, or death presumed to be cardiac in origin. In conclusion, this study found that pimobendan significantly delayed the median time to a primary endpoint (1228 days vs. 766 days for the placebo group).[239] Additionally, the heart size of dogs in the pimobendan decreased with treatment.[240] This study led to FDA approval of pimobendan, marketed as a separate formulation (Vetmedin®-CA1), for dogs with stage B2 cardiac disease.[211] There is also evidence that pimobendan is beneficial for delaying the onset of clinical signs in Doberman Pinchers with preclinical DCM.[171]

Interest in treating cats with pimobendan is growing even though the most common cause of CHF in cats is Hypertrophic Cardiomyopathy (HCM).[241] HCM is a condition where inotropic

therapy is contraindicated because of the diastolic failure and/or left ventricular outflow tract obstruction associated with this disease.[242] Nonetheless, because the prognosis for HCM cats in CHF is poor, there is a desire to find therapies that could increase survival time and/or quality of life.[243] Study results are conflicting as to whether pimobendan increases survival time for cats in CHF from HCM. A retrospective case-control study found that cats with HCM and CHF receiving pimobendan had a significant increase in survival time (626 days vs. 103 days in case-controls).[244] Yet, a prospective, randomized study following 83 cats with HCM and recent, controlled CHF found no benefit for pimobendan and outcome at 180 days.[245]

Pharmacokinetics of pimobendan in veterinary species

Pharmacokinetics (PK) is a branch of clinical pharmacology dedicated to studying the rate of and relationships between absorption, distribution, metabolism, and excretion of a drug.[246] The pharmacokinetics of pimobendan in healthy dogs has been investigated in intravenous and oral formulations.[247-250] Using different two different oral formulations at the Vetmedin® label dose (0.25 mg/kg), the reported mean maximum plasma concentrations (C_{max}) of pimobendan ranged from 18.6 ng/mL to 42.96 ng/mL in dogs.[250-252] For the studies that measured plasma concentrations of ODMP, the ODMP C_{max} values were comparable to the C_{max} values of pimobendan.[251,252] Interestingly, the package insert for Vetmedin® lists a C_{max} of 3.09 ng/mL and 3.66 ng/mL for pimobendan and ODMP, respectively.[227] The reason for this discrepancy is unknown but could be because of differences in the analytic technique used.[250] One study reported an oral bioavailability of ~70% for Vetmedin® capsules.[250] The Vetmedin® product insert states that food decreases the bioavailability of an aqueous solution, but the effect on the tablet form is unknown.[227] Recently, the population pharmacokinetics of pimobendan in dogs

with subclinical and clinical MMVD (Stages B2, C or D) was studied.[251] This study found a similar mean C_{max} of pimobendan and ODMP (42.96 ng/mL and 39.65 ng/mL, respectively) compared to previous dog PK studies, however, the PK parameters were highly variable in these dogs, especially the values for pimobendan absorption and elimination rate. The PK variability was not associated with stage of heart disease or any other clinicopathologic variable measured.

Two studies have investigated pimobendan PK in healthy cats following oral administration.[253,254] At approximately 0.25 mg/kg doses, the mean C_{max} values of pimobendan obtained in these studies were 34.5 ng/mL and 45.4 ng/mL. One of the studies also measured plasma ODMP concentrations in the study cats.[254] Interestingly, the area under the concentration-time plot extrapolated to infinity ($AUC_{0-\infty}$) of pimobendan was higher than the $AUC_{0-\infty}$ of ODMP in these cats, which suggested that cats have reduced conversion of pimobendan to ODMP.

Singular studies have investigated the PK of pimobendan in rabbits and parrots, respectively.[255,256] The study performed in New Zealand White rabbits used a mean dose of 2.08 mg/kg, as it was difficult to accurately administer lower volumes of Vetmedin® tablets. The rabbits had a lower mean C_{max} (15.7 ng/mL), longer time to maximum concentration, and longer half-life compared to pimobendan PK in dogs and cats. The authors hypothesized that this was because of differences in gastrointestinal tract anatomy and physiology between rabbits and monogastric species[255] The study performed in Hispaniolan Amazon parrots found that even at a dose of 10 mg/kg, the parrots had low (mean C_{max} 8.26 ng/mL) and erratic plasma concentrations of pimobendan.[256]

Pimobendan in horses

Little is known about the efficacy of pimobendan in horses. Anecdotally, it has been used in horses with CHF.[68] One study investigated the cardiovascular effects of a single dose (0.25

mg/kg) of pimobendan administered intragastrically (i.g.) or intravenously (i.v.) to healthy mature horses.[257] In this study, five mares were instrumented with a central venous pressure jugular catheter and an intraarterial carotid catheter for measurements of direct blood pressure and cardiac output (via lithium dilution). Using a balanced Latin square design, the horses were administered Vetmedin tablets®, pimobendan injectable solution, or water (placebo). Cardiovascular variables were measured at baseline and at five additional timepoints up to eight hours. Results showed a significant percentage increase in heart rate after administration of i.g. and i.v. pimobendan compared to the placebo (33± 4% (mean ± standard deviation), 36 ± 14%, and -2 ± 7%, respectively). After i.v. administration only, the horses had a significant percentage decrease in right ventricular end diastolic pressures (EDP), suggesting decreased preload. Again after i.v. administration only, a variable used to evaluate contractility (maximum rate of increase in pressure (dP/dt_{max}) had a significant percentage increase compared to the placebo, suggesting increased inotropy. The authors concluded that pimobendan administered i.v. to healthy horses results in chronotropic, inotropic, and vasodilatory effects. The authors speculated that the reason for increased but not statistically significant changes in dP/dt_{max} seen after i.g. administration of pimobendan was due to possible poor oral bioavailability of pimobendan in horses, citing that horses have poor oral absorption of many drugs well absorbed orally in other species.[183,258,259] While this study showed promising results of i.v. pimobendan in horses, pimobendan and ODMP plasma concentrations were not measured in this study, therefore, further studies of the bioavailability and pharmacokinetics of pimobendan in horses are warranted.[178]

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Chapter 3: Single Dose Pharmacokinetics of Pimobendan in Healthy Horses

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Abstract:

Pimobendan is an inodilator approved for treatment of canine cardiac disease. The pharmacokinetics have not been investigated in horses. This study evaluated the pharmacokinetics of oral Vetmedin® (V) in horses. Additional objectives were to determine the equivalence of compounded pimobendan capsules (C) and suspension (S) and the effects of sample site on plasma drug concentrations. Six horses received a single 0.5mg/kg dose of pimobendan via oral syringe. A pilot study with two horses was performed, these horses received C, S, or V using a crossover design with a minimum 1-week washout period. Plasma samples were collected simultaneously from lateral thoracic and jugular catheters before and after drug administration at predetermined times. The remaining 4 horses received V only with jugular samples collected. Differences between formulation and sample site were analyzed by one-way ANOVA. Analysis was by LC-MS/MS and noncompartmental pharmacokinetics for V. No significant differences were noted between formulations or sample site ($P > 0.05$). Concentrations in compounded formulations were 90%(C) and 88%(S) of label. For V, mean (±SD) maximum plasma concentration (Cmax) was 4.96 ± 2.13ng/mL at 2.17 ± 0.98hr, and area under the curve (AUC0–∞) was 22.1 ± 8.8hr*ng/mL. The active metabolite, o-desmethyl-pimobendan, was not detected in any sample (<0.07ng/mL). At 0.5mg/kg PO, pimobendan

plasma concentrations were considerably lower than reported in dogs. There was no evidence of oral transmucosal absorption. Pimobendan was poorly absorbed in horses, regardless of formulation, and appears unlikely to have clinical effects.

1. Introduction:

Pimobendan is a non-sympathomimetic, non-glycoside inodilator with positive inotropic effects via calcium sensitization and phosphodiesterase (PDE) 3 inhibition, and vasodilatory effects also through PDE 3 inhibition [1,2]. Pimobendan is converted to its active metabolite, O-desmethyl-pimobendan (ODMP), in the liver[3], and species differences in the amount of metabolite produced are known to exist[4].

Pimobendan is a cornerstone of therapy for myxomatous mitral valve disease (MMVD), dilated cardiomyopathy, and congestive heart failure (CHF) in dogs[5-8]. The pharmacokinetics of oral pimobendan has been extensively studied in dogs[9-11], and has also been investigated in cats[4,12], rabbits[13], and parrots[14]. Studies evaluating the pharmacodynamics of pimobendan in dogs have illustrated that pimobendan improves indices of cardiac output[8,10,15]. Additionally, studies examining the outcomes of dogs in CHF receiving pimobendan have shown an overall increase in survival time[6,7], and pimobendan is now recommended to delay the onset of CHF in dogs with stage B2 (nonclinical) MMVD[16].

Valvular disease—particularly mitral valve regurgitation—is frequently seen in mature horses[17,18]. While mild to moderate valvular regurgitation typically carries a good to fair prognosis, the progression of this disease in horses is not well studied[19,20]. Therefore, the risk and rate of progression of mild to moderate valvular insufficiency to congestive heart failure in horses in unknown, and there is minimal evidence available on appropriate treatments to slow progression of disease.

One small study has investigated the effects of a single dose of intragastric (i.g.) and intravenous (i.v.) pimobendan (0.25 mg/kg) in healthy horses[21]. That study showed that horses had a significant percent increase in heart rate via either route, but only i.v. administration produced significantly decreased end diastolic pressures and significantly different rates of change in right ventricular minimum and maximum pressures compared to the placebo group. Because no statistically significant inotropic effects were seen with i.g. administration, the authors concluded that pimobendan might have poor oral bioavailability in horses. However, no pharmacokinetic data was collected during this study. Overall, the results of this study suggest that pimobendan has positive cardiac effects when given to healthy mature horses.

The objective of this study was to describe the pharmacokinetics of a single dose of orally administered pimobendan in healthy mature horses. Additionally, a subset of horses was used to determine the bioequivalence of compounded pimobendan products compared to the canine FDA approved formulation of pimobendan (Vetmedin®), as well as the effect of sample site collection on plasma drug concentrations to evaluate the potential for oral transmucosal absorption of pimobendan in horses. The equivalence of compounded formulations was investigated because these formulations have convenience factors, including ease of administration (e.g. lower volume of drug, more palatable to horses) and accessibility when Vetmedin® is not available due to backorder of the product. The hypothesis of this study was that pimobendan, as Vetmedin®, would be absorbed in horses when administered at a 0.5 mg/kg dose orally, at concentrations similar to those found to be therapeutic in other species.

2. Methods:

2.1 | Animals

Six healthy, privately-owned adult horses were enrolled in the study. There were five mares and 1 gelding, ranging in age from 16 to 24 years old (mean age 17 years) and weighing 365.9 to 565.9 kg (mean weight 452.3 kg). Breeds represented included 2 Arabians, and one each of Thoroughbred, Quarter Horse, Tennessee Walking Horse, and Draft Cross. The horses were determined to be healthy by physical examination and a serum biochemistry panel before starting the study. The serum biochemistry analysis was performed by the Virginia Tech Animal Laboratory Services. The study was approved by the Virginia Tech University Institutional Animal Care and Use Committee (Protocol 22-244) and informed owner consent was obtained.

2.2 | Drug administration

A pilot study was performed using two horses in a randomized three-way crossover design with a minimum one-week washout period. The horses were administered either compounded pimobendan capsules (10 mg/capsule), compounded pimobendan oil-based suspension (10 mg/mL), or Vetmedin® (10 mg tablets) orally at a 0.5 mg/kg dose. The remaining four horses received Vetmedin® tablets only at the same dose after the pilot study was completed.

The contents of the pimobendan capsules were dissolved in water and the Vetmedin® tablets were crushed in a clean blender (nutribullet®). No modification was necessary for the pimobendan suspension. The capsules or suspension were then mixed with approximately 20 mL of water and 20 mL of Karo® dark corn syrup in a dosing syringe (60-cc catheter-tipped syringe) immediately prior to oral administration. For the Vetmedin® tablets, a larger volume of water (approximately 120-150 mL) was needed to appropriately mix the crushed tablets in a dosing

syringe, which required the use of four to five 60-cc catheter-tipped syringes depending on the number of tablets.

The drug was administered orally on the opposite side of the mouth from the external jugular catheter. The individual administering the drug was not the same individual that performed the blood sample collection. For all portions of the study, the horses were fasted for at least 10 hours before drug administration and housed in individual stalls. The horses were provided ad libitum orchard-timothy grass hay beginning 2 hours post-drug administration.

2.3 | Sample collection

In the pilot study, a 16-gauge over-the-wire catheter (Long-term MILACATH® Guidewire Introduction Kit) was placed in a lateral thoracic vein of each horse concurrently with a 14-gauge catheter (Mila International, Inc, Florence, KY, USA) in one external jugular vein. Both catheters were placed using aseptic technique. During the pilot study's final phase, an exception occurred as a lateral thoracic catheter could not be placed in one of the horses likely due to moderate thrombophlebitis of the vessels. Therefore, no lateral thoracic plasma sample data for that horse receiving Vetmedin® tablets was obtained. Blood samples were collected simultaneously from both catheters immediately prior to drug administration (time 0) and at time 15, 30, and 45 min, and 1 ,2, 4, 8, 12, and 24 h post administration. Prior to sample collection, waste blood (8 ml for jugular catheters, 6 mL for lateral thoracic catheters) was withdrawn and discarded. Immediately after sample collection (6 ml) the catheters were flushed with 10 mL of heparinized saline. Blood samples were collected into lithium heparin blood tubes and centrifuged at 3600rpm (1160 g) for 5 minutes. The plasma was then transferred into cryovials and stored at -80°C until analysis of drug concentrations were performed.

The results of the pilot study did not support any beneficial effect of the compounded formulations on drug absorption, nor any significant effect of sample site on plasma concentrations. Therefore, the final four horses used in the study received Vetmedin® tablets only, with samples described as above only from a jugular vein catheter.

2.4 | Analysis of compounded formulations

Upon receipt, the compounded pimobendan capsules and oil-based suspension were stored at room temperature (22-25°C) in light-proof containers as recommended by the compounding pharmacy. To determine the strength of the compounded products, the contents of one capsule or 1 mL of suspension were dissolved in 100% methanol to a target concentration of 1.1 mg/mL and 1 mg/mL, respectively. The solutions were further diluted in mobile phase and analyzed using the assay described below.

2.5 | Analysis of blood samples

Plasma samples were analyzed at the VA-MD College of Veterinary Medicine Analytical Chemistry Research Laboratory for pimobendan and ODMP concentrations using UPLC-MS/MS.

Reference standards of pimobendan and ODMP were obtained from Cayman Chemical and Key Organics, respectively. Isotopically labeled internal standards of pimobendan-d3 (Pd3) and O-desmethylpimobendan-d4 (ODMP-d4) were obtained from Toronto Research Chemicals, and Sussex Research Labs, respectively. Stock solutions of all compounds and internal standards were initially made up in dimethyl sulfoxide (DMSO) and then separately diluted in methanol (MeOH) + 1% formic acid (FA) to their final standard concentrations.

Plasma standards and samples were prepared using protein precipitation by adding 150 µL of plasma to 300 µL of the internal standard addition solution (15 ng/mL Pd3+Od4 in MeOH + 1% FA) in 2 mL polypropylene (PP) microcentrifuge tubes. The samples were briefly shaken, vortexed for 5 minutes, then centrifuged at 16,100 g for 5 minutes. The supernatant was then transferred to 2 mL amber autosampler vials with low volume inserts.

Sample extracts were subjected to chromatographic separation performed on a Waters H-Class UPLC system with an HSS T3 reverse phase column (Waters Acquity UPLC HSS T3, 100 mm length x 2.1 mm ID x 1.8 µm) and matching guard column (Waters Acquity UPLC HSS T3 VanGuard Pre-Column, 5 mm length x 2.1 mm ID x 1.8 µm) maintained at 40°C. Five microliters of sample were injected onto the column using a refrigerated autosampler maintained at 5 °C. Mobile phase A consisted of 1% FA in water (H2O), mobile phase B consisted of 1% FA in acetonitrile (ACN), and mobile phase C was 100% MeOH. The mobile phase was delivered to the UPLC column at a flow rate of 0.4 mL per min, using a gradient elution program. The retention times of the pimobendan/Pd3 and ODMP/Od4 were approximately 1.00 and 1.40 minutes, respectively. The compounds were detected using triple-quadrupole mass spectrometer (Waters Xevo TQD) equipped with a Zspray ionization source operated in positive-ion electrospray mode (ESI+) with multiple reaction monitoring (MRM).

A calibration curve (0.4 – 100 ng/mL) was prepared in blank equine plasma in the same manner as the samples. The concentration of analytes in the standards and samples was computed based on the sample/IS AUC ratio. Using this assay, the limit of quantification was 0.233 ng/mL based on a signal to noise (S/N) ratio of 10, and the limit of detection was 0.04 ng/mL based on a S/N ratio of 3 for both analytes. Interday accuracy and precision were calculated using 5 replicates at 0.4, 12, and 40 ng/mL. For pimobendan, accuracy was 105%,

97.6% and 99.4% and precision was 2.25%, 2.4% and 0.91% at the low, medium and high concentrations, respectively. For ODMP, accuracy was 104%, 95.1%, and 99.3% and precision was 2.87%, 2.42% and 2.04% at the low, medium and high concentrations, respectively.

2.6 | Pharmacokinetic and statistical analysis

Noncompartmental analysis (NCA) was performed on plasma pimobendan concentrations using commercially available computer software (Phoenix WinNonlin, version 6.3, Certera, Raleigh, NC). Maximum serum concentrations (C_{max}) and time to maximal serum concentration (T_{max}) were obtained directly from the plasma concentration data. The calculated pharmacokinetic parameters obtained from the model were the terminal-phase rate constant (λz), half-life ($T_{1/2}$), the area under the curve from time 0 to infinity ($AUC0\rightarrow\infty$), and the extrapolated percentage of the area under the curve (AUC %).

For statistical analysis of differences between plasma concentration following administration of different formulations and collection from different sample sites, the data was first checked for normality using the Shapiro-Wilk test. All data was found to be non-normally distributed, so was further analyzed using the Kruskal-Wallis One Way Analysis of Variance (SigmaPlot ver 14.0, Grafiti LLC, Palo Alto, CA, USA). Significance was set at a P value ≤ 0.05.

3. Results

Pimobendan was detected in the plasma of all horses following administration of all 3 formulations studied. No significant differences were noted between formulations (Figure 1) or sample site (Figure 2) in the two initial horses. Pimobendan concentrations in the compounded formulations were 0.994 ± 0.049 mg/mL and 0.878 ± 0.02 mg/mL representing a mean of 90% and 88% of label concentration for the capsules and suspension, respectively.

In horses administered Vetmedin®, drug was initially detected at 15 minutes after administration in 3/6 horses, and 30 minutes after administration in the remaining 3 horses. Concentrations were above the LOQ for 8 hours in all 6 horses (Figure 3). Relevant pharmacokinetic parameters for pimobendan following administration of 0.5 mg/kg Vetmedin® are presented in Table 1. ODMP was below the limit of detection in all samples (<0.07ng/mL).

Parameter	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	Mean ± SD
C_{max} (ng/mL)	6.17	6.32	3.05	2.29	7.77	4.14	4.96 ± 2.13
T_{max} (hr)	2.00	4.00	1.00	2.00	2.00	2.00	2.17 ± 0.98
λ_z (hr^{-1})	0.49	0.54	0.39	0.41	0.40	0.47	0.45 ± 0.06
$T_{1/2\,\lambda z}$ (hr)	1.40	1.28	1.79	1.68	1.72	1.47	1.56 ± 0.2
$AUC_{0-\infty}$ (hr*ng/mL)	21.01	30.12	14.54	10.84	33.85	22.17	22.1 ± 8.8
AUC_{extrap} (%)	3.21	1.38	5.00	4.89	5.35	2.82	3.78 ± 1.56

Table 1. Pharmacokinetic data from six horses receiving a single 0.5 mg/kg PO dose of Vetmedin® C_{max} = maximum concentration; T_{max} = time to maximum concentration; λz = slope of the terminal phase; T ½ λz = half-life of terminal phase; $AUC_{0-\infty}$ = area under the concentration-time curve extrapolated to infinity; AUC_{extrap} = percent of $AUC_{0-\infty}$ extrapolated.

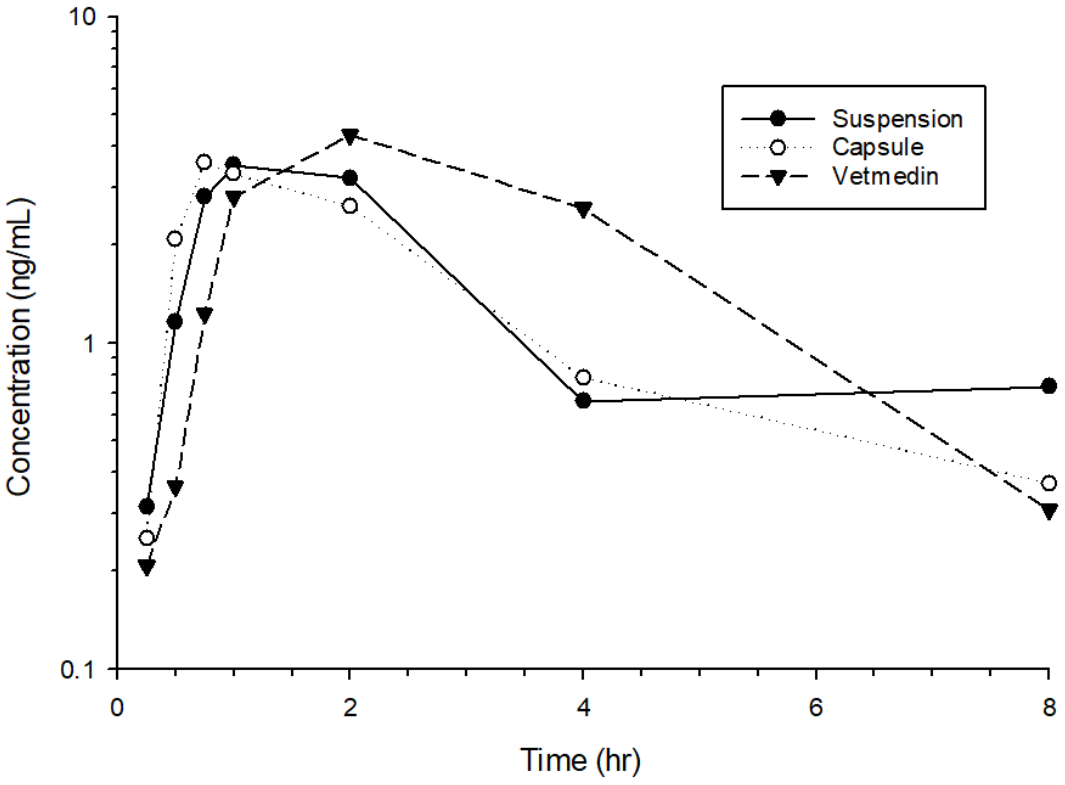

Figure 1. Mean jugular concentration vs. time curve for three formulations of pimobendan when administered as a single dose at 0.5 mg/kg PO (n = 2). Each circle or triangle represents the mean plasma concentration for the two horses at various time points (15, 30, 45 min, 1, 2, 4 and 8 hours). The differences between formulations were not statistically significant (horse #1 p = 0.70, horse #2 p = 0.95).

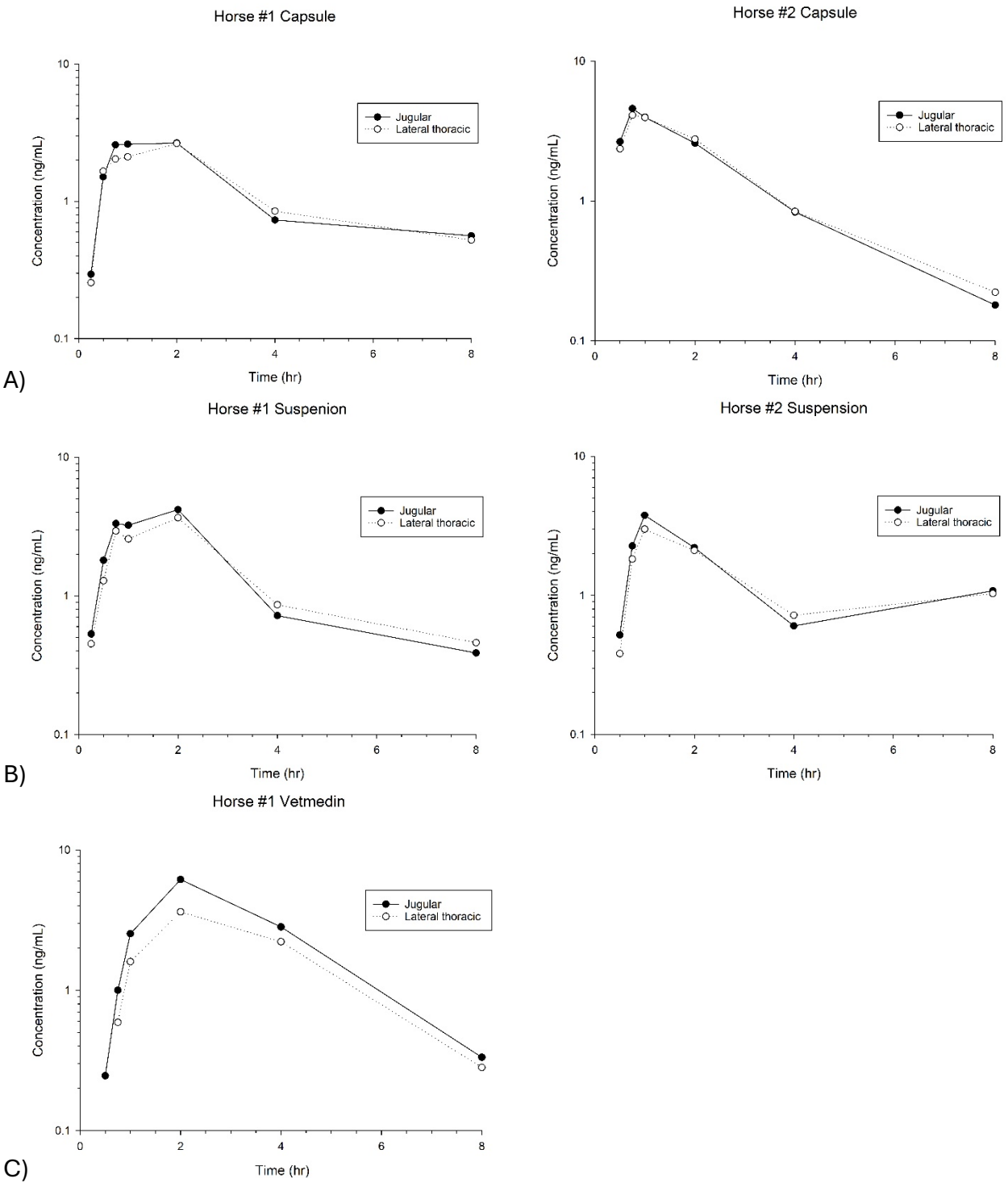

Figure 2. Comparison of plasma concentration versus time curves for pimobendan administered orally as a compounded capsule (row A, n=2), compounded suspension (row B, n=2), or Vetmedin® tablets (C, n=1) when sampled from the jugular vein catheter versus lateral thoracic

vein catheter. The differences between sample sites were not statistically significant (capsules: horse #1 p = 0.85, horse #2 p = 0.97; suspension: horse #1 p = 0.65, horse #2 = 0.85; Vetmedin®: horse #1 p = 0.87).

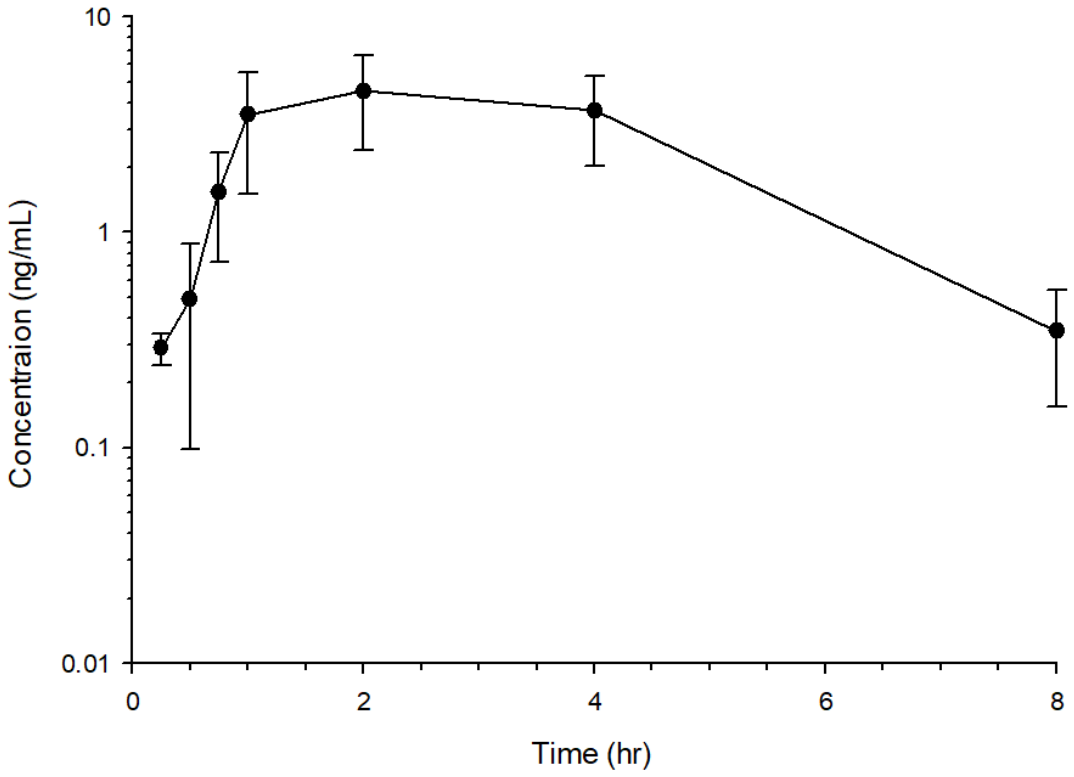

Figure 3. Concentration vs. time curve for the mean plasma concentrations of pimobendan after administration of a single dose of Vetmedin® at 0.5 mg/kg PO (n = 6). Each circle represents the mean plasma concentration at time points 15, 30, and 45 min, 1, 2, 4 and 8 hours, and the error bars represent the standard deviation.

4. Discussion

The results of this study indicate that oral absorption of pimobendan is low in horses, and a dose of 0.5 mg/kg orally is unlikely to result in cardiac effects, based on known therapeutic

concentrations in other species. These findings do not support previous findings that pimobendan administered enterally to horses at 0.25 mg/kg has chronotropic effects. The mean C_{max} value in horses (4.96 ± 2.13 ng/mL) was approximately $1/10^{th}$ the average C_{max} value (49.1 ± 28.7 ng/mL) reported in a canine pharmacokinetic study that used the same dose (0.5 mg/kg) and formulation (Vetmedin® tablets)[22]. Other studies using lower doses (0.25 mg/kg and 0.36 mg/kg) of Vetmedin® still attained much higher mean C_{max} values in dogs (38.1 ± 18.3 and 42.96 ng/mL, respectively) than the average C_{max} value obtained in this study[9,11]. The pharmacokinetics of pimobendan has been investigated in other species, with cats and rabbits demonstrating different mean C_{max} values than dogs (Table 2). Interestingly, investigators in a rabbit study[13] used an average Vetmedin® dose that was eight times higher than the dose recommended for dogs[23], and found that the average C_{max} values for the rabbits were less than half of the average C_{max} value obtained in canine studies. Rabbits and horses are hind-gut fermenters, and due to this distinct anatomical difference from monogastric species (i.e. cats and dogs), there is likely substantial differences in their pharmacokinetic profiles[24]. Furthermore, horses are known to have poor enteral absorption of drugs that are well absorbed enterally in dogs[25,26].

Oral transmucosal absorption (OTM) occurs with some drugs and can result in falsely elevated plasma concentrations when sampling via the jugular vein. This typically manifests as a very high C_{max} occurring at the first sampling time point. Studies in horses that use oral administration (as opposed to intragastric administration via nasogastric intubation) should evaluate for potential OTM absorption by analyzing drug concentrations from the jugular and comparing those to a vein at distant site. Based on the low C_{max} and prolonged T_{max} for the 2 horses in the pilot study, OTM absorption of pimobendan is unlikely to contribute to a significant portion of the plasma concentrations reported.

Study	Her et al.[22]	McManamey et al.[11]	Hanzlicek et al.[12]	Yata et al.[4]	Ozawa et al.[13]	Jula et al. (this study)
Dose	0.5 mg/kg	0.36 mg/kg#	0.28 mg/kg*	0.28 mg/kg#	2.08 mg/kg*	0.5 mg/kg
Species	Dog	Dog	Cat	Cat	Rabbit	Horse
Assay	HPLC	HPLC	HPLC-MS	UHPLC-MS/MS	LC- MS/MS	UPLC-MS/MS
C_{max} (ng/mL)	49.1 ± 28.7	42.96	34.5	45.4 (41.4-70.9)	15.7 ± 7.54	4.96 ± 2.13
T_{max} (hr)	2.1 ± 0.9	1.66	0.9	0.3 (0.3-1.0)	2.79 ± 1.25	2.17 ± 0.98
T1/2 (hr)	1.8 ± 0.8	0.97@	1.3@	0.7 (0.5-1.1)	3.54 ± 1.31	1.56 ± 0.2
$AUC_{0-\infty}$ (hr*ng/mL)	148.4 ± 71.6	197.11	95.2	89.9 (66.3 – 160.5)	65.11 ± 18.85	22.1 ± 8.8

Table 2. Results from previous studies investigating the pharmacokinetics of pimobendan in dogs, cats, and rabbits. Vetmedin® tablets were administered orally in all studies. Data from Yata et al. is reported as median (range). The remainder of the data is reported as mean ± standard deviation. # = median dose administered, * = mean dose administered, @ = elimination half-life

The active metabolite of pimobendan, ODMP, was below the LOD (0.07 ng/mL) in all samples. This is relevant to the effectiveness of pimobendan in horses, as ODMP has greater PDE 3 inhibition compared to its parent compound[27]. PDE 3 inhibition contributes to the inotropic effects of pimobendan[28]. A study performed on isolated canine right ventricular myocardium found that ODMP was 500 times more potent than pimobendan in increasing the

force of myocardial contraction[29]. Canine pharmacokinetic studies demonstrate that ODMP plasma concentrations closely mimic pimobendan plasma concentrations,[15,22] however cats have reduced conversion of pimobendan to ODMP[4]. It is not possible to speculate based on the data obtained in this study whether horses lack the ability to metabolize pimobendan to ODMP, or if the inability to detect ODMP is due to the poor oral absorption of pimobendan in horses.

In this study, the two compounded formulations of pimobendan had similar absorption compared to the reference formulation (Vetmedin®), with no significant differences noted between plasma concentration versus time profiles in the two horses that received all three formulations (Figure 1). Several studies investigating the pharmacokinetics of compounded pimobendan have been published in dogs[9,10,30]. These studies demonstrated that the compounded pimobendan formulations had satisfactory bioavailability and pharmacodynamics (when measured) compared to the reference drug. However, stability of pimobendan in compounded aqueous formulations has been questioned[31]. The desire for efficacious compounded formulations of pimobendan stems from the need for formulations to accurately dose small dogs, and the fact that Vetmedin® shortages have occurred in the United States in recent years[32]. For the horses in this study, the compounded formulations had the advantage of being easier to administer than Vetmedin® tablets as the volumes administered were smaller. Because of the smaller volumes, we were able to add Karo® syrup to the compounded formulations to increase palatability, which was not done for the Vetmedin® tablets due to the large volume. Dosing of Vetmedin® tablets at 0.5 mg/kg required the 10 mg tablets to be mixed with a considerable amount of water to achieve delivery through a 60-cc oral syringe, and therefore four to five syringes were required for each horse. Nonetheless, because compounded drugs do not undergo consistent testing for identity, quality, strength, purity and stability, results of research described

in reports using compounded products may not be reproducible. Therefore, the Vetmedin® formulation was used in the remaining horses after the pilot study. While this study provides preliminary evidence that oral absorption of the compounded formulations used in the present study is similar to the FDA-approved formulation for dogs, data was only available for 2 animals, and it is unknown if absorption would be similar for other compounded formulations.

The current study was not without limitations. These limitations include the absence of intravenous pimobendan to determine true bioavailability, possible loss of drug during administration due to the route of administration, and the small number of horses used in the study. Injectable pimobendan is not currently available in the United States, although it is produced in other countries (Vetmedin® injectable solution for dogs, 0.75 mg/mL, Boehringer Ingelheim, Ingelheim am Rhein, Germany). Administration to the horses in this study was considered cost prohibitive and compounded intravenous products were not used due to concern over sterility. Pimobendan formulations were delivered via oral syringe rather than nasogastric intubation to more closely resemble clinical use. However, despite care to ensure complete administration, this likely led to small amounts of the drug failing to be swallowed by the horses, particularly the Vetmedin® tablets which the horses notably disliked.

In summary, after oral administration, pimobendan plasma concentrations in horses were considerably lower than what is reported in dogs. There was no evidence of oral transmucosal absorption of the drug in the pilot study. Further research is needed to determine if pimobendan at higher doses is absorbed adequately in horses to warrant therapeutic use. However, higher doses of the only FDA approved formulation, Vetmedin®, would be impractical to administer to horses in the currently available tablet sizes. Pimobendan at 0.5 mg/kg is poorly absorbed in horses, regardless of formulation, and appears unlikely to have clinical effects.

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Chapter 4: Final Comments

The results of this study showed that oral pimobendan was poorly absorbed in horses, nullifying our hypothesis that it would produce plasma concentrations in horses akin to concentrations found to be therapeutic in dogs. The mean C_{max} value in these horses (4.96 ± 2.13 ng/mL) was approximately 1/10th the average C_{max} value reported in a canine pharmacokinetic study (49.1 ± 28.7 ng/mL) that used the same dose and formulation of pimobendan. This finding may be related to anatomical differences in the gastrointestinal tracts of these species, as horses tend to have poor oral absorption of drugs that are well absorbed in monogastric animals.

Pimobendan has revolutionized treatment of myxomatous mitral valve disease (MMVD) in dogs, delaying onset to clinical signs of congestive heart failure (CHF) and prolonging the lifespan of dogs with CHF. Mitral valve regurgitation secondary to an analogous MMVD process is very prevalent in the horse population. Therefore, investigation of pimobendan at higher doses or with different formulations may be warranted in horses. However, the concentration of drug in the currently available tablet sizes of Vetmedin®, the FDA approved formulation of pimobendan, makes higher dosing impractical to near impossible in horses.

Ultimately, oral administration of pimobendan to horses at the dose used in this study, 0.5 mg/kg, is unlikely to be efficacious and is not recommended for extra-label use in horses with CHF. Nonetheless, further studies of pimobendan at higher doses are warranted before concluding that this drug does not have utility in horses.