Orally administered torsemide in horses: pharmacokinetic and pharmacodynamic studies

by

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Abstract

Diuretic therapy is the mainstay for management of congestive heart failure in horses. Loop-diuretic medications in horses have been restricted to injectable medications because currently, no data support the use of orally-administered loop diuretics.

The objectives of this study were to determine the pharmacokinetic and pharmacodynamic properties of the orally-administered, loop-diuretic torsemide and determine if it could be used as an alternative to injectable diuretics in the horse. A total of 6 healthy adult mares were used in a 2-phase, prospective study. The two phases consisted of the pharmacokinetic profiling of a single dose (6 mg/kg PO) and pharmacodynamic effects of long-term torsemide administration (2 mg/kg q12h) for 6 days in healthy horses. Pharmacokinetic analysis identified a peak concentration ($C_{\text{max}}$) of 10.14 µg/mL (range, 6.793-14.69 µg/mL) and elimination half-life ($T_{1/2}$) 9.205 hours (range, 8.383-10.43 hours). The area under the plasma drug concentration over time curve (AUC) was 80.7 µg * h/mL (range, 56.5-117.2 µg * h/mL). A statistically significant increase in urine volume and decrease in urine specific gravity were found between day 0 (baseline) and day 6 ($p<0.0001$). Significant alterations in biochemical variables included hyponatremia, hypokalemia, hypochloremia, and increased serum creatinine concentration. Mean arterial blood pressure significantly decreased on day 6 (57.67±8.814 mmHg, $p=0.001$) as compared to baseline (78±6.132 mmHg). Serum aldosterone concentrations significantly increased after 6 days of torsemide administration ($p=0.0006$).
Orally-administered torsemide (4 mg/kg/day) successfully reached therapeutic concentrations in blood, induced clinically relevant diuresis, and resulted in moderate pre-renal azotemia and electrolyte disturbances.
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List of Abbreviations

CHF congestive heart failure
ECFV extra cellular fluid volume
EIPH exercise induced pulmonary hemorrhage
ICFV intracellular fluid volume
MAP mean arterial pressure
RAAS renin-angiotensin-aldosterone system
TBW total body water
USG urine specific gravity
Chapter 1 – Literature review

Section 1: Definition and pathogenesis of fluid overload and edema formation

Fluid overload is characterized by hypervolemia with expansion of the vascular space, and as a consequence, distribution of the extra fluid to the interstitium. Edema is the consequence of the redistribution of this extra fluid, either within cells (cellular edema) or within the collagen-mucopolysaccharide matrix distributed in the interstitial spaces (interstitial edema).

Physiologic fluid spaces are mainly divided between the total body water (TBW), extra cellular fluid volume (ECFV), and intracellular fluid volume. Considering that the body fluid spaces are a representation of a physiologic and dynamic measurement (percentage of body weight), obtained at a specific time point, TBW can be estimated to be 2/3 of the body weight. The remaining 2/3 of the total body water volume is comprised by fluid found within the cells, the intracellular fluid volume (ICFV). Results from most of the studies evaluating TBW in the horse, suggests that a healthy horse has a volume of TBW between 60 and 70% of its weight. Approximately 1/3 of the TBW is comprised of the volume of fluid contained outside of the cells. Plasma volume expansion as seen in fluid overload can lead to edema; whereas plasma volume contraction can lead to shock. Accurate clinical monitoring of plasma volume through packed cell volume and total solids
assessment can be challenging in the horse, mostly due to interfering factors such as splenic contraction and abnormal protein loss. Serial packed cell volume and total solids provide a good estimative of a trend which can be beneficial in determining plasma volume expansion, and contraction.\(^2\) Generally, edema occurs within the extracellular fluid space, although it can also take place in the intracellular fluid compartment.\(^6\)

Although edema formation is a physiologic effect with certain positive consequences such as an increase of convective flux of macromolecules (e.g. complement proteins and antibodies used to aid in killing of bacteria within the interstitial space), and dilution of certain cytotoxic chemicals released by microorganisms, edema also is closely related to negative consequences.\(^1\) The edematous tissue often has a decrease in oxygenation and nutrients supply due to an increase in diffusion distance, and also impaired nutritive tissue perfusion with the collapse of capillaries (especially in encapsulated organs and tissues with decreased expansion capability of the interstitial volume such as the kidneys and bones).\(^1\)

Fluid overload and edema formation can occur with various conditions in the horse including iatrogenic fluid overload \(^2\), renal and congestive heart failure (CHF)\(^2\), pulmonary edema \(^7\), distal limb cellulitis \(^8\), head trauma \(^9\), and respiratory distress syndrome in foals. \(^10\) The induction of excessive hypervolemia in healthy horses, leading to fluid overload, is unlikely given that horses can tolerate a high volume of intravenous fluid over a short period of time (approximately
80mL/kg/h).\textsuperscript{3} Just by gravity alone, with the use of a regular 14 gauge intravenous catheter, the administration of a fluid volume high enough to induce fluid overload is unlikely in the horse.\textsuperscript{2} Nevertheless, according to a previous study, in vitro administration of a high fluid volume can be achieved in a short period of time with the use of fluid pumps, larger gauge catheters, and multiple intravenous catheters.\textsuperscript{11}

Fluid overload frequently arises with abnormal renal function and inability to excrete the excess of water. Fluid overload can occur with normal renal function in the presence of other risk factors such as: systemic inflammation, increase in capillary permeability, changes in interstitial compliance, alteration in lymphatic outflow, and hypoproteinemia, which ultimately interfere with the kidneys’ ability to recognize the extra fluid to be excreted.\textsuperscript{2} According to Ernest Starling, in most capillaries, a balance or near-equilibrium is present in a way that the amount of fluid being filtered outward from an arterial capillary, is almost the same as the amount of fluid returned to the circulation via the lymphatics.\textsuperscript{6} Ernest Starling proposed that four primary forces are important determinants of which direction fluid will move (i.e. either from the intravascular space to the interstitium or in the opposite direction).\textsuperscript{6} Summarizing the Starling forces, it can be stated that the net filtration pressure is determined by difference between the capillary pressure and the interstitial fluid pressure, subtracted by the difference between capillary plasma colloid pressure and the interstitial fluid colloid pressure. The simplified Starling Force equation and model of fluid
movement from the intravascular space towards the interstitial compartment are represented in figure 1.

Net filtration = \( K_f \left[ (P_{\text{cap}} - P_{\text{int}}) - (\pi_{\text{plasma}} - \pi_{\text{int}}) \right] \)

(Adapted from Equine Fluid Therapy)\(^2\)
Figure 1. The model represents the Starling forces directing the fluid movement from the intravascular space towards the interstitium. The hydrostatic pressure with the capillary is referred as $P_{\text{cap}}$. The hydrostatic pressure within the interstitium is represented by $P_{\text{int}}$. The plasma oncotic pressure is referred as $\pi_{\text{plasma}}$, whereas the interstitial fluid oncotic pressure is referred as $\pi_{\text{int}}$.  

This equation was revised by Levick and Michel (2010), which added a coefficient ($K_f$) for a presumed increase in permeability of the capillary wall during inflammation.\(^1\)\(^2\) Net filtration is the sum of all the forces involved in the equation. The intravascular hydrostatic pressure is normally generated by the heart. In horses with congestive heart failure, this hydrostatic pressure is increased and, as a consequence, there is a movement of the extra fluid from the intravascular space towards the interstitial compartment.\(^2\) It is consensus that edema will take place whenever there is a disruption of that continuous system, leading to movement of the fluid from the intravascular space towards the interstitial space and to the lymphatics. In summary, interstitial edema can occur when the plasma oncotic pressure is reduced from decreased protein production or protein loss; in cases of congestive heart failure with an increase in capillary hydrostatic pressure; in the face of inflammation with an increase in capillary permeability; and with a decrease in lymphatic drainage in cases of lymphatic obstruction or when lymph nodes are surgically removed.\(^2\)
Section 2: Renal physiology – movement of water and solutes

The kidneys play an important role in the regulation of water balance, the management of fluid overload, and edema formation. As previously mentioned in Section 1, water movement through capillaries towards the interstitium follow a sum of forces (hydrostatic and colloid osmotic forces), whereas the movement of fluid between the intracellular and extracellular compartments is closely related to the osmotic effect of the solutes. The composition of the extracellular fluid is meticulously regulated by the kidneys, which have an imperative role in allowing the cells to be bathed in a fluid with proper concentration of solutes and nutrients for optimal cell function and metabolism.6

The kidneys have a variety of physiologic functions such as regulation of water and electrolytes, metabolism, secretion and excretion of hormones, as well as excretion of metabolic waste products and foreign chemicals. Also, the kidneys have an important role in regulation of body fluid osmolality and electrolytes concentration, regulation of blood pressure and acid-base balance, as well as other vital functions such as regulation of erythrocyte production and gluconeogenesis.6 In order to better understand the role of water and salt retention in the perpetuation of edema, it is important to review the normal renal physiologic events in regards to solute filtration and reabsorption by the kidneys. The nephron is the functional unit of the kidney,
and a simplified diagram of its segments, percentage of solutes secreted and reabsorbed, as well as the site of action of the different diuretics available is demonstrated on figure 2.

The nephron is composed of the glomerulus, the proximal convoluted tubule, the thin descending limb of the loop of Henle, the loop of Henle, the thin ascending limb of the loop of Henle, the thick ascending limb of the loop of Henle, the distal convoluted tubule, the cortical collecting duct and the medullary collecting duct. The kidney has two types of nephrons: the cortical nephron which is superficial and has a short loop of Henle, and the juxtamedullary nephron with the glomerulus site at the cortical region and with a long loop of Henle which extends deep into the medullary region of the kidney. Overall, the equine nephron has a similar morphologic composition when compared to other species. Two capillary arteries (afferent and efferent) provide the glomerulus with control of vasoconstriction or vasodilation, which helps dictate the filtration rate. The glomerular capillaries are mainly under influence of high hydrostatic pressure (approximately 60 mm Hg), allowing a rapid fluid filtration. In contrast, the peritubular capillaries are under the influence of a lower hydrostatic pressure (approximately 13 mm Hg), which facilitates a rapid fluid reabsorption. Therefore, the urine formation and its constituents, are a product of the difference between glomerular filtration and tubular reabsorption and secretion.

Factors that affect the glomerular filtration include: molecule size, shape, and also the charge of the particles that are being filtered by the glomerulus. Approximately 20% of the total blood will
be filtered by the glomerulus, and a much higher permeability of the glomerular capillary bed to ions when compared to other capillary membranes in the body, constitute a filtrate with a high concentration of ions that will later be mostly reabsorbed by the renal tubules.

Approximately 65-70% of the water, sodium, and chloride will be reabsorbed by the proximal renal tubules. This portion of the nephron has a high rate of water and solute reabsorption that is performed mainly via a passive, isosmotic, paracellular process. This portion of the renal tubule also reabsorbs all the low-molecular-weight solutes that are essential for cell function, such as amino acids and glucose. The proximal tubule similarly regulates other solutes such as potassium, calcium, inorganic phosphate, magnesium, bicarbonate and hydrogen. Although the exact percentage of potassium reabsorption by the proximal renal tubules in the horse is unknown, this is the major site for potassium recovery in the equine nephron, and is estimated to have a potassium reabsorption of approximately 55-70%. Most of the inorganic phosphate is reabsorbed within the proximal renal tubules (approximately 80%). The proximal tubule is responsible for only 5-20% of the reabsorption of magnesium, a process that is performed via a paracellular transport and requires water diffusion. Approximately 70% of the body magnesium is filtered by the glomeruli with the majority being reabsorbed in different segments of the nephron. Sodium movement from the tubular lumen to the cell also occurs via a counter-transport mechanism that reabsorbs sodium while secreting hydrogen, an important process in
acid-base regulation that facilitates bicarbonate reabsorption.\textsuperscript{6,13} Approximately 80\% of the bicarbonate filtered by the glomerulus is reabsorbed by the proximal tubules.\textsuperscript{13}

The loop of Henle, as previously mentioned, is divided in segments which have different physiologic properties in regards to fluid and solute movement. The descending part of the thin segment is highly permeable to water and moderately permeable to most solutes. This segment of the nephron allows mainly simple diffusion with approximately 15-20\% of water being reabsorbed in this segment.\textsuperscript{6} The ascending limb (including both the thin and thick portions) is almost impermeable to water. This lack of permeability to water in the ascending limb of the loop of Henle is essential for urine concentration. The reabsorption of sodium, potassium and chloride in the thick ascending limb of the loop of Henle is approximately 25\%. This segment is next major site for reabsorption of solutes and is an active form of transport that is performed by the sodium-potassium-chloride (Na/K/2Cl) cotransporter, present in the luminal membrane of the thick ascending limb.\textsuperscript{15} The major site for reabsorption of magnesium is the thick ascending limb of the loop of Henle. Approximately 50-70\% of the magnesium reabsorption occurs in this segment of the nephron and is performed by a paracellular transport, driven by the positive voltage gradient generated when sodium, chloride, and potassium are reabsorbed by the Na/K/2Cl cotransporter.\textsuperscript{13} About 20\% of the calcium is also reabsorbed in this segment by the same transportation process as magnesium.
The distal segment of nephron and the collecting duct are responsible for secretion of solutes and also reabsorption of a minimal amount of solutes. Approximately 8-10% of sodium and chloride are reabsorb within the distal tubule and collecting duct.\textsuperscript{2} The distal convoluted tubule reabsors approximately 10% of the filtered magnesium. Beyond this point, there is minimal reabsorption of magnesium; therefore the collecting tubules are responsible for determining the final concentration of magnesium in urine.\textsuperscript{13,17} Although acid secretion occurs in all segments of the nephron, secretion of ions hydrogen via an active transport performed by the intercalated cells occurs in the distal and collecting tubules. This acid secretion facilitates reabsorption of potassium and bicarbonate.\textsuperscript{13} The distal tubule is not only responsible for potassium reabsorption but also for its secretion. Potassium is actively secreted in the distal tubule lumen by the direct influence of aldosterone in the principal cells.\textsuperscript{15} The final concentration of urine is also determine by additional water reabsorption that takes place in the collecting ducts. This process is mediated by vasopressin and is facilitated by specific aquaporin channels in the collecting ducts.\textsuperscript{6,13}

Fluid overload with edema formation is often a result of an inability of the kidneys to recognize the excess water or a failure to excrete it.\textsuperscript{2} One example of the connection of edema and renal function can be observed in patients with edema secondary to congestive heart failure. In the presence of heart failure, a decrease in stroke volume raises the venous pressure causing an increase in capillary filtration.\textsuperscript{6} A subsequent decrease in renal perfusion due to a decrease in arterial pressure is also observed leading to less excretion of salt and water, perpetuating the
edema. As a consequence for this decrease in renal blood flow, the renin-angiotensin-aldosterone system (discussed later in Section 5) is activated, leading to additional retention of water and salt.\textsuperscript{6}
Figure 2. Diagram representing the nephron with the different diuretics available and their respective site of action in the nephron.

http://www.pathophys.org/diuretics/
Section 3: History of diuretics and their mechanism of action

Diuretics are among the most used medications in human medicine, and most of the diuretic drugs act by decreasing the reabsorption of sodium and chloride at different sites of the nephron (Figure 2). As a consequence of this decreased reabsorption of solutes, there is an increase in urinary sodium excretion, and subsequent increase in water loss.

Diuretic therapy has been extensively used for the treatment of edema, however its effective use does not go too far back in the medical history. Treatment of dropsy (i.e. edema), which for a long time was centered in increasing body secretions or mechanical removal of excess fluid, was extremely frustrating and ineffective until the beginning of the 19th century. Edema was referenced in ancient medicine texts from Sumeria, Egypt, Greece and Rome, with great focus but different etiologies and empirical therapies. As an example of the importance of edema and the necessity for an effective therapy, the ancient Sumerian word for physician was “Azu”, meaning “One Who Knows Water”.

The first effective diuretic described was discovered by Paracelsus in the year of 1520. Paracelsus treated dropsy with success with the use of an inorganic form of mercury, calomel, which its use became limited due to serious gastrointestinal side effects. The 20th century’s first effective diuretics were from an organic form of mercurial. A Viennese medical student named Alfred Vogel,
noticed that merbaphen, a newly introduced organic mercurial agent used for the treatment of syphilis, increased the urinary output in patients. Organic mercurial agents were used as the major diuretic therapy for the following 20 years after its discovery by Alfred Vogel. However, the toxic gastrointestinal side effects related to long-term use of organic mercurials lead to investigation and discovery of a new diuretic class, the carbonic anhydrase inhibitors. This class of diuretics was discovered by Schwartz in 1949, who demonstrated diuretic effect in three patients with refractory congestive heart failure after administering a new antimicrobial with carbonic anhydrase inhibitor activity, sulfanilamide. In 1954, acetazolamide was synthetically produced and distributed as the first carbonic anhydrase inhibitor to replace organic mercurials in the treatment of edema secondary to congestive heart failure. Acetazolamide works by inhibiting the carbonic anhydrase enzyme activity in the proximal tubule, therefore decreasing the availability of ions hydrogen for the sodium-hydrogen exchanger. As a consequence, there is a reduction of sodium reabsorption and impaired neutralization of luminal bicarbonate with an increase reabsorption of ions chloride. The result is a hyperchloremic metabolic acidosis with very mild diuresis produced, likely due to the fact that the fluid leaving the proximal tubule gets reclaimed in the other segments of the nephron. The minimal diuresis promoted and the significant metabolic acidosis induced, limited the use of acetazolamide. In 1955, exploration of a sulfonamide derivative that could mobilize edema with the excretion of sodium along with chloride instead of bicarbonate, lead to the synthesis of chlorothiazide. Although thiazides are also carbonic anhydrase inhibitors, the main sites of action is in the cortical portion of the loop of Henle.
and the distal convoluted tubule. This type of drug inhibits the sodium and chloride reabsorption by competing directly with the chloride binding site and inhibiting the sodium-chloride symporter. Thiazides, as well as decreasing sodium reabsorption within the distal tubule, also increase the calcium reabsorption in this segment of the nephron.

In 1959 loop diuretics were developed, and furosemide was released for clinical use in 1964. Loop diuretics, also known as high ceiling diuretics, acquired their class name due to the site of action: the ascending thick limb of the loop of Henle. A variety of chemical agents are included within this class (furosemide, bumetanide, and torsemide), and they work by blocking the Na/K/2Cl cotransporter in the luminal membrane. Furosemide binds competitively to the chloride binding site of the luminal Na/K/2Cl cotransporter. This portion of the loop of Henle, in contrast to the thin descending limb, is mainly impermeable to water. Consequently, by blocking the Na/K/2Cl cotransporter, it increases the sodium, chloride, and potassium delivery to the distal tubule, thereby inducing the production of large quantities of isotonic urine. Loop diuretics, along with a great diuretic effect, induce a significant potassium loss in urine, which prompted the research and development of an alternative for a diuretic agent with less potassium wastage. In 1961, the pharmaceutical industry developed spironolactone, a diuretic drug that would block potassium secretion at the distal tubule by inhibiting the reabsorption of sodium at this segment of the nephron.
Spironolactone has aldosterone antagonist effects, and is a potassium sparing diuretic. Spironolactone works by competing with the receptor site of aldosterone in the late distal tubule, therefore inhibiting the reabsorption of sodium and water, and increasing the reabsorption of potassium. Since a minimal percentage of sodium is normally reabsorbed by the distal tubule, the amount of diuresis induced by spironolactone is much lower when compared to loop diuretics. Therefore, loop diuretics are currently the most potent diuretics and the mainstay for fluid overload control and management of critical cases requiring diuresis.

Section 4: Renin-angiotensin-aldosterone system and its activation by diuretics

The Renin-angiotensin-aldosterone system (RAAS) is an endocrine system extremely important in the regulation of blood pressure and extracellular fluid volume by controlling intravascular volume, cardiovascular function, and the renal excretion of water and electrolytes. Renin is an enzyme that is produced by the juxtaglomerular cells of the kidney and released into the systemic circulation. There are a variety of physiologic events that trigger renin production and release: renal artery hypotension with a decrease pressure in the afferent arteriole, sympathetic stimulation with stimulus of beta-1 adrenergic nerves, and a decrease in sodium concentration at the distal tubule detected by the macula densa mechanism. Although plasma renin activity has been shown to be low in the horse when compared to other species, a response of aldosterone to activation of the RAAS seems to be appropriated in the horse, with an increase in plasma...
aldosterone noted in response to decrease in plasma volume. Once in the systemic circulation, renin cleaves hepatic angiotensinogen to angiotensin I, which is converted to angiotensin II in the lungs by an angiotensin-converting enzyme. Consequently, angiotensin II binds to its receptor in the adrenal cortex and stimulates the release of aldosterone to increase renal sodium and water reabsorption within the collecting duct. Aldosterone simultaneously increases renal sodium reabsorption and potassium secretion.

Loop diuretics activate the RAAS by decreasing plasma volume, which is an undesirable effect since the RAAS acts as an antagonist of loop diuretics by stimulating the retention of water and sodium by the kidney. Continuous activation of RAAS due to long-term administration of loop diuretics may lead to diuretic resistance. In a study performed in healthy dogs, plasma aldosterone concentrations were significantly increased from baseline after long-term furosemide and torsemide administration. In the same study, long-term administration of torsemide markedly increased 24 hour urine volume, whereas furosemide induced only modest diuresis, suggesting that long-term administration of torsemide might lead to less diuretic resistance despite an increase in plasma aldosterone concentration.
Section 5: Loop diuretic therapy in the horse and other species

Loop diuretics (e.g., furosemide, torsemide) have been used for clinical conditions in horses, humans, dogs and cats to remove excessive fluid from the body by blocking the Na-K-2Cl transporters in the thick ascending loop of Henle. Once the active reabsorption of sodium, potassium, and chloride is blocked, diuresis is promoted by excretion of water, sodium, potassium, and chloride. Intravenously administered furosemide is commonly used to resolve pulmonary edema secondary to acute CHF, whereas orally-administered diuretics are the mainstay for successful long-term management of CHF.\textsuperscript{29,30} Diuretics also are indicated to increase urine output in horses with acute renal failure, oliguria, anuria, poisoning, envenomation, and severe hypercalcemia or hyperkalemia.\textsuperscript{10,31-33}

In the horse, diuretic therapy is restricted to injectable formulations of furosemide because of a lack of current data to support the use of oral furosemide. Frequent intramuscular or intravenous administration of furosemide can lead to complications such as local infection, perivascular administration of the drug, thrombosis and accidental injection into the carotid artery.\textsuperscript{34} A pharmacokinetic and pharmacodynamic study of oral furosemide in healthy horses indicated that the bioavailability of furosemide using a single dose of 1 mg/kg was only 5%.\textsuperscript{35} In the same study, no differences in urine volume and plasma electrolyte concentrations were noted after oral furosemide administration when compared to control horses.
Torsemide is a high ceiling loop diuretic that is 10 times more potent than furosemide, and has a consistently high bioavailability after oral administration in humans and dogs. Torsemide has been widely used in humans and dogs with CHF, and shown less diuretic resistance. Although torsemide is proven to be safe and effective with good absorption after oral administration in humans, dogs, cats and rats, pharmacokinetic and pharmacodynamic studies have not been reported in horses.

The absorption of orally-administered torsemide is nearly complete in humans, dogs and rats. A formulation of torsemide for oral administration was rapidly absorbed in human patients with CHF, and similar results were observed in a pharmacokinetic study of torsemide in healthy human volunteers and patients with renal failure.

**Section 6: Pharmacological advantages of torsemide and study justification**

Torsemide has several pharmacological advantages over furosemide. In a study comparing diuretic effects of equivalent dosages of torsemide and furosemide in small animals, less potassium loss was observed in animals treated with torsemide. In humans, torsemide has high bioavailability which allows an immediate switch from IV to PO formulation without changing the dosage. A half-life that allows dosing every 12 hours, good absorption after oral administration, and low risk of diuretic resistance are potential advantages of orally-administered torsemide for long-term
diuretic therapy, which may contribute to clinical and prognostic improvement in patients with fluid overload.

In a pilot study performed by this research team, torsemide was administered orally (6mg/kg, every 12 hours for 4 days, followed by 12mg/kg, every 12 hours for additional 4 days) to an 15 year old Thoroughbred stallion with congestive heart failure and monitored for 8 days. At day 7 a significant decrease in the ventral edema was noted by subjective visualization (Figure 3); also, a decrease in venous congestion was observed. No evidence of azotemia was observed (Figure 4). Daily plasma concentration showed an increase in torsemide plasma concentration levels that were directly correlated with dosage (Figure 5). Electrolyte measurements revealed mild hyponatremia and hypochloremia and moderate hypokalemia. A moderate hypokalemia (1.9 mmol/L; reference range: 3.5 – 4.5 mmol/L) was observed at day 4 (Figure 4) of treatment, and potassium was supplemented by oral administration of potassium chloride (KCl) at a dose of 0.1 g/kg, twice a day.
Figure 3. Marked improvement of pectoral ventral edema and jugular venous congestion in a stallion with congestive heart failure after oral torsemide administration. (A) Severe ventral edema (arrow) at Day 0, (B) Substantially reduced ventral edema at Day 7 (arrow), (C) Pronounced jugular distention at Day 0 (arrowhead), (D) Absent jugular distention at Day 7 (arrowhead).

Figure 4. Changes in potassium (A) and creatinine (B) levels over the course of oral torsemide administration in a stallion with CHF.
Preliminary results indicated that torsemide has good palatability and was safe to be administered orally in horses. Also, the high plasma torsemide concentration levels and improvement in clinical signs along with evidence of electrolyte changes indicated that torsemide was absorbed after oral administration and was effective in managing this patient with congestive heart failure.

The mainstay therapy of congestive heart failure in the horse is based on diuretic therapy, and the most commonly used diuretic in horses, furosemide, is not well absorbed orally. Owner compliance, duration of treatment, and the skill to administer injectable furosemide may limit the
effectiveness and the feasibility for long-term management of congestive heart failure in the horse. The purpose of this study is to determine if a more powerful loop diuretic, torsemide, may be used as an oral alternative for diuretic therapy in horses. We hypothesized that torsemide would be well-absorbed when administered PO, and produce clinically relevant diuresis in healthy horses. The study aims were to determine the pharmacokinetic profile after single PO administration of torsemide and to evaluate the pharmacodynamic effects of long-term administration of torsemide PO in healthy horses. To our knowledge, this study constitutes the first pharmacokinetic and pharmacodynamic investigation of orally-administered torsemide in the horse.
Chapter 2 – Pharmacokinetic profile of orally administered torsemide in healthy adult horses

The aims of this study phase was to determine the plasma drug concentrations and pharmacokinetic profile of torsemide after oral administration. The hypothesis was that torsemide would reach therapeutic concentrations in blood when administered orally.

Section 1: Materials and methods

Animals and criteria

Six healthy, adult mares (5 quarter horses and 1 draft cross horse) from the teaching herd of the Auburn University Large Animal Teaching Hospital were enrolled. Their average body weight and age were 594 kg (range, 477-541 kg) and 15 years (range, 11-23 years), respectively. All horses were used in a 2-phase study with a minimum of 90 days wash-out period between phases. All horses were deemed clinically healthy based on physical examination, baseline echocardiography, indirect blood pressure, CBC, plasma fibrinogen concentration and results of serum biochemical profiles. This experiment was approved by the Institutional Animal Care and Use Committee of Auburn University.
Plasma torsemide concentration determination

Plasma concentrations of torsemide were determined by a high performance liquid chromatography (HPLC) method as previously described. The HPLC equipment included Agilent 1100 series pumps, temperature controlled auto-injection, ultraviolet detector, and interface. A C-18 column (Phenomenex; 2.6 u; 100x4.6 mm; Torrance, CA) was used with a mobile phase of acetonitrile and potassium dihydrogen orthophosphate buffer (0.05M) at a ratio 40:60 by volume. Detection was at 290 nm and the flow rate was 1 mL/min. To 1 mL of equine plasma sample or plasma standard, 0.8 mL of 10% perchloric acid in methanol was added and the samples were centrifuged at 1048 x g. A 20 uL volume was injected into the HPLC system. The assay was evaluated in plasma with respect to recovery, linearity, selectivity, limit of quantification, precision and accuracy. Standards were prepared with equine plasma by spiking with aliquots of torsemide to yield concentrations of 0.01, 0.04, 0.1, 0.4, 1.0, 4.0, 10.0 and 20.0 µg/mL. Plasma standards stored for 4.5 months at -80°C showed no evidence of drug degradation. Drug concentrations were determined from equine plasma standard curves based on a peak area. The limit of quantification was 0.04 µg/mL and the limit of detection was 0.01 µg/mL. Intra- and inter-day variations were < 5.5%.
Experiment phase I (pharmacokinetic profiles)

Each horse was stalled for 3 days (1 day acclimatization and 2 days of blood collection). Before torsemide administration (6mg/kg, PO, once), a 14-gauge IV catheter was aseptically placed in the left jugular vein for blood collection. Access to hay and water was restricted for 2 hours before and 4 hours after the single dose administration of torsemide. Thereafter, free choice hay and water was offered. Torsemide tablets were crushed, mixed in 500 mL of water, and administered via a nasogastric tube (Figure 6). The tube was irrigated with 500 mL of water and removed after drug administration. Ten mL of blood was collected into lithium heparin tubes to measure plasma torsemide concentrations. Blood was collected before treatment (T=0), and at times 5, 10, 20, 30, 45, 60 minutes, and 2, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours after administration. Blood was centrifuged for 10 min at 1200 rpm, and the supernatant plasma was harvested and stored at -80ºC for a total of 3 days before batch analysis.
Figure 6. Torsemide administration at 6mg/kg, via nasogastric tube (A), followed by 500 milliliters of water via nasogastric tube (B).

Pharmacokinetic data analysis

Torsemide pharmacokinetic parameters were estimated by non-compartmental analysis using Phoenix WinNonlin Ver. 7 (Pharsight). The terminal decrease in the natural log plasma concentrations was used to calculate the excretion to elimination rate constant (λz) and the t1/2
was determined as $0.693/\lambda z$. The AUC and its first moment (AUMC), were assessed by the linear/log trapezoidal rule method. The apparent total body clearance after PO administration ($\text{Cl/F}$), the apparent volume of distribution ($V_{\text{area/F}}$), and mean residence time (MRT) were estimated from individual values of AUC, AUMC, and $t_{1/2}$. Pharmacokinetic data were expressed as mean, median, geometric mean, standard deviation (SD), and coefficient of variation (% CV).

Section 2: Results

Pharmacokinetic profile of torsemide after a single PO dose (Phase I)

Torsemide was absorbed after intragastric administration with a wide kinetic variability among individuals (Figure 7). Average duration to peak plasma concentration ($T_{\text{max}}$) was approximately 3 hours (range, 1.75-4 hours) (Table 1). Peak torsemide plasma concentration ($C_{\text{max}}$) was 10.14 µg/mL (range, 6.793-14.69 µg/mL). The excretion half-life of the terminal phase ($T_{1/2}$) was 9.2 hours (range, 8.38-10.43 hours). The area under the plasma drug concentration over time curve (AUC) was 80.7 µg * h/mL (range, 56.5-117.2 µg * h/mL).
Figure 7. Mean plasma concentrations over time following a single intragastric administration with 6 mg/kg of torsemide in 6 healthy adult mares. Bars represent standard deviation.
Table 1. Pharmacokinetic Parameters after oral torsemide administration (6 mg/kg)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median (µg/mL)</th>
<th>25% Percentile</th>
<th>75% Percentile</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>10.1</td>
<td>6.79</td>
<td>14.6</td>
<td>11.0</td>
<td>5.26</td>
<td>47.6</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>3</td>
<td>1.75</td>
<td>4</td>
<td>2.83</td>
<td>1.33</td>
<td>46.9</td>
</tr>
<tr>
<td>AUC (µg ⋅ h/mL)</td>
<td>80.7</td>
<td>56.5</td>
<td>117</td>
<td>92.2</td>
<td>48.9</td>
<td>53</td>
</tr>
<tr>
<td>λ (1/h)</td>
<td>0.07</td>
<td>0.06</td>
<td>0.08</td>
<td>0.07</td>
<td>0.01</td>
<td>18</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>9.20</td>
<td>8.38</td>
<td>10.4</td>
<td>9.27</td>
<td>1.65</td>
<td>17.8</td>
</tr>
<tr>
<td>V&lt;sub&gt;area/F&lt;/sub&gt; (L/kg)</td>
<td>0.89</td>
<td>0.74</td>
<td>1.56</td>
<td>1.08</td>
<td>0.55</td>
<td>51.3</td>
</tr>
<tr>
<td>Cl/F (L/h/kg)</td>
<td>0.07</td>
<td>0.05</td>
<td>0.10</td>
<td>0.07</td>
<td>0.02</td>
<td>37.8</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>11.5</td>
<td>9.16</td>
<td>14.3</td>
<td>11.6</td>
<td>2.53</td>
<td>21.8</td>
</tr>
</tbody>
</table>

C<sub>max</sub>, maximum concentration; T<sub>max</sub>, time of maximum concentration; AUC, area under the concentration-time curve; λ, slope of the terminal phase; T<sub>1/2</sub>, half-life of the terminal phase; V<sub>area/F</sub>, apparent volume of distribution; Cl/F, apparent total body clearance; MRT, mean residence time.
Chapter 3—Pharmacodynamic properties of long-term oral administration of torsemide to healthy adult horses

The aims of this study phase was to evaluate the pharmacodynamic effects of torsemide after prolonged oral administration. The hypothesis was that torsemide would induce clinically significant and persistent diuresis after prolonged oral administration, elicit a significant decrease in stroke volume, and induce mild electrolyte derangements.

Section 1: Materials and methods (Phase II)

The 6 horses employed for the phase I study were used in the phase II study after a wash-out period of 90 days. Each horse was stalled for 8 days (1 day acclimatization and 7 days of data collection). The tablets (100 mg/tablet) were crushed and mixed with 60 mL of water and administered PO at a dosage of 4 mg/kg/day for 6 days. Horses had access to free choice water, free choice hay, and 1.4 kg of a 10% pelleted feed once daily throughout the study period. All horses were weighed daily. A 28 Fr Foley urinary catheter was aseptically placed into each mare’s bladder and connected to a urine collection bag (Figure 8). Urine bags were maintained using a truss so that the mares could move freely in the stall (Figure 9). Urine was collected over the course of 12 hours on day 0 (before drug administration), and on days 1 and 6 (after drug administration). Urinary catheters were removed after 12 hours of urine collection. Collection bags were emptied
hourly, and urine specific gravity (USG) and volume were measured. Blood samples were collected at 8 am daily for determination of packed cell volume (PCV), total protein concentration (TP) and serum biochemical profiles. Mean arterial pressure (MAP) was measured with a tail cuff using an oscillometric monitor device (SurgiVet® Advisor) as previously described. Mean arterial pressure was measured daily at T=0 (before drug administration), and 1, 5, and 9 hours after drug administration. Echocardiographic parameters were evaluated by a board-certified cardiologist (SWJ), using a digital cardiovascular ultrasound imaging system (Philips® HD-11) with a S3-1 (3-1 MHz) cardiovascular transducer. Standard long- and short-axis views were used to obtain echocardiographic images. Parameters measured included mitral and tricuspid inflow pattern, chamber dimensions of the left ventricle and the left atrium, trans-aortic flow velocity, trans-pulmonary flow velocity and left ventricular volume measured by a modified Simpson’s method. All echocardiographic data obtained was normalized to body weight as previously described. Echocardiography was performed on day 0 (baseline) and then on day 6 of torsemide administration. Samples for plasma aldosterone concentration were obtained daily 1 hour after torsemide administration in the morning. Plasma aldosterone concentrations were measured by radioimmunoassay (Coat-A-Count Aldosterone Radioimmunoassay) as previously validated for horses.
Figure 8. Urine collection bag used for continuous urinary collection.
Figure 9. Urinary bags maintained using a truss in order to allow free movement in the stall during urinary collection.

Statistical analysis

Assessment of data distribution in regard to normality was determined using the Kolmogorov-Smirnov test. Normally distributed variables were expressed as mean ± SD. A Student’s t-test with Bonferroni correction was used to analyze differences in pharmacodynamic parameters between
day 0 (baseline before torsemide administration) and day 6 of torsemide administration. Central
tendency and dispersion for non-normally distributed data were expressed as median and
interquartile range (IQR). A 1-way analysis of variance was used to determine differences between
pharmacodynamic variables across different time points: day 0 (before drug administration), day
1 (first day of torsemide administration) and day 6 (last day of torsemide administration). If a
statistically significant difference was detected among 3 time points, a Tukey’s test was used to
compare differences between 2 time points. A commercial software package was used for all
statistical analysis, and a corrected p-value < 0.05 was set as statistically significant.

Section 2: Results (Phase II)

Pharmacodynamic profiles after torsemide given PO for 6 days (Phase II)

Urine volume, urine specific gravity and body weight

Oral torsemide administration significantly changed USG, and no statistical difference was
observed in body weight when compared between baseline (day 0; before torsemide
administration) and day 6 (Figure 10). A significant increase in urine volume (median; IQR) was
noted: day 0 (2204; 1881-3874 mLs), day 1 (26665; 23185-31225 mLs), and day 6 (11525; 9140-
14170 mL). A statistically significant increase in urine volume occurred from day 0 to day 6
(p<0.0001). Urine specific gravity (median; IQR) significantly decreased after torsemide treatment (day 0: 1.046; 1.033–1.048) and (day 1: 1.010; 1.009–1.011p<0.0001).
**Figure 10.** (A) Total urine volume, (B) Urine Specific Gravity, and (C) Body Weight (kg) in each horse before and after oral torsemide administration with 2 mg/kg q12h at three different time points (day 0, circle; day 1, squares; day 6, triangles). The asterisk indicates statistical significance (corrected p-value < 0.05).

**Plasma electrolyte, BUN, creatinine, total protein, and plasma aldosterone concentrations, and PCV (%)**

Serum electrolyte, BUN, creatinine, and TP concentrations, and PCV were significantly altered by the prolonged administration of torsemide (**Figure 11 and 12**). Hyponatremia (median; IQR) was noted on day 6 with a significant decrease in serum sodium concentration (129; 128.8-131.0 mmol/L; p<0.0001) from baseline (137; 136.8-139.5 mmol/L). Similarly, hypochloremia was observed on day 6 (79.1±3.4 mmol/mL; p<0.0001) from baseline (101.2±1.7 mmol/L). Moderate hypokalemia was noted on day 6 (3.7±0.2 mmol/L; P<0.0001) when compared to baseline (1.9±0.2 mmol/L). Metabolic alkalosis was noted with a significant increase in bicarbonate concentration on day 6 (32.3±3.8 mmol/L; p<0.0003) from baseline (23.2±1.1 mmol/L). Azotemia was noted with a significant increase in serum creatinine concentration (baseline, 1.4 mg/dL±0.1; day 6, 2.0±0.1 mg/dL) and BUN (baseline, 14.5±1.8 mg/dL; day 6, 23.5±6.0 mg/dL; p<0.0001 and p=0.0058, respectively). A significant increase in TP was noted on day 6 (7.5±0.5 g/dL, p<0.0130) from baseline (6.6±0.3 g/dL). Increased PCV was noted after long-term torsemide administration
(baseline, 35±2%; day 6, 43±3%; p=0.0005). A significant increase was identified in plasma aldosterone concentrations over the course of torsemide administration (p=0.0006): day 0 (14.6±9.6 pg/mL), day 1 (59.2±38.2 pg/mL) and day 6 (100.2±27.5 pg/mL) (Figure 13).
Figure 11. Serum electrolytes before and after oral torsemide administration with 2mg/kg q12h at day 0 and 6. (A) Sodium; (B) chloride; (C) potassium; (D) The asterisk indicates statistical significance between the different time points (corrected p-value < 0.05).
Figure 12. Biochemistry changes, total protein, and packed cell volume before and after oral torsemide administration with 2mg/kg q12h at day 0 and 6. (F) creatinine; (G) total protein; (H) packed cell volume. The asterisk indicates statistical significance between the different time points (corrected p-value < 0.05).
Figure 13. Serum aldosterone concentrations before and after oral torsemide administration with 2mg/kg q 12h at 3 different time points (day 0, circle; day 1, squares; day 6, triangles). A significant increase in aldosterone levels was noted when comparing the baseline (day 0) with day 1 and with day 6. No statistical difference was observed between day 1 and day 6. The asterisk indicates statistical significance (corrected p-value < 0.05).

Echocardiography and mean arterial pressure

The effects of PO torsemide on echocardiographic variables and MAP are shown in figures 13 and 14. Normalized left atrium maximal diameters were significantly decreased on day 6 (1.5; 1.4-1.5,
Mean arterial pressures decreased significantly on day 6 (57 ± 9 mmHg, \( p=0.001 \)) as compared to baseline (78±6 mmHg).

**Figure 14.** Normalized echocardiographic parameters before and after oral torsemide administration with 2mg/kg q 12 hours, at day 0 and 6. (A) Left atrial diameter, LAD; (B) mitral valvular annulus, MVA, (C) left ventricular internal diameter at end-diastole, LVIDd; (D) left
ventricular internal diameter at end-systole, LVIDs; The asterisk indicates statistical significance between the different time points (corrected p-value < 0.05).
Figure 15. Non-invasive stroke volume measurements and non-invasive mean arterial systolic blood pressure before and after oral torsemide administration with 2mg/kg q 12 hours, at day 0 and 6. (E) Stroke volume measured by a modified Simpson’s method, MOD SV; (F) Bullet stroke volume, Bullet SV; (G) Teichholz stroke volume, Teichholz SV; (H) non-invasive mean arterial systolic blood pressure. The asterisk indicates statistical significance between the different time points (corrected p-value < 0.05).
Chapter 4: Discussion

Orally-administered torsemide administration caused significant diuresis with an increase in urine volume and a decrease in USG in all horses. Results also indicated that torsemide reached therapeutic concentrations in blood, resulting in persistent diuresis and changes in biochemical and hemodynamic test results.

Torsemide has less diuretic resistance and anti-aldosterone effects in dogs. Although bioavailability could not be calculated in our study because of unavailability of a torsemide formulation for IV use, it was absorbed after PO administration in all horses, reaching plasma concentrations similar to those observed in previous studies in dogs, rabbits, rats and humans. Our results showed that after a single dose of 6 mg/kg, plasma torsemide concentration reached a median Cmax of 10.14 µg/mL and time to reach peak plasma concentration was approximately 3 hours. In humans, peak plasma concentrations are obtained approximately 1 hour after PO torsemide administration and in dogs, peak plasma concentrations were observed at 1.5 hours after drug administration. For the single PO torsemide dose of 6 mg/kg, plasma torsemide concentrations over time showed great variability, and could not be fitted to a compartmental model. Plasma concentration of torsemide after a single PO dose was variable among individuals over the initial 14 hours with an AUC that had a high CV (53%). The high variability for torsemide concentration among individuals and the late onset of peak plasma concentration...
concentration in our study could be explained by several factors. The nature and amount of ingesta in the gastrointestinal tract of each horse could have played a role and explained the different onset of peak plasma concentration and also the differences in drug absorption (e.g., drug binding to ingesta). All horses had hay and water restricted for 2 hours before and 4 hours after the single PO dose administration of torsemide (Phase I), and then free choice hay and water were offered. It is likely that the amount of ingesta in the gastrointestinal tract of each horse was different, which could have interfered with drug absorption. The influence of diet, anatomy, and physiology of the equine gastrointestinal tract in the absorption of orally-administered torsemide is unknown. However, in humans, simultaneous administration of food with torsemide led to a decrease in drug absorption as indicated by a decrease in Cmax and a delay in reaching peak plasma drug concentration with no influence on total drug bioavailability and diuretic effects. In our study, a single PO dose of torsemide resulted in an elimination half-life 3 times longer than what was observed in humans and slightly shorter than what was observed after IV torsemide administration in dogs. The terminal elimination half-life is influenced by drug clearance, but plasma drug decay also can be influenced by the tissue distribution rate. Based on the low CV% for the termination half-life (17.8%), it is likely that the variation among individuals was caused by drug absorption and clearance. Although the CV was high (37.8%), PO torsemide administration resulted in a low apparent total body clearance with a mean of 0.07 L/h/kg, and this finding could be the reason why PO torsemide showed a relatively long terminal half-life in our study.
Another important factor is that the pharmacokinetic data reported in our study was obtained from healthy individuals. Physiologic changes seen in CHF, such as a decrease in mesenteric and portal blood flow and increased sympathetic and decreased parasympathetic activity, may have a negative influence on drug absorption, distribution and clearance.\textsuperscript{52} Despite this, absorption of orally-administered torsemide seems to be similar in healthy and diseased individuals. In a previous pharmacokinetic study, PO and IV formulations of torsemide resulted in similar plasma concentrations in patients with CHF, reaching C\textsubscript{max} in 1 hour after administration and an absolute bioavailability of 89\% was observed.\textsuperscript{40} These results indicate that the pharmacokinetics of torsemide in patients with CHF are comparable with those in healthy individuals.\textsuperscript{8} Similar results were obtained in a pharmacokinetic study of torsemide in healthy volunteers and patients with renal failure.\textsuperscript{41} As previously reported in humans, furosemide has considerable variability with regard to its absorption, which makes it difficult to predict how much furosemide will be absorbed by a given patient. In contrast, the absorption of torsemide in humans, dogs and rabbits is nearly complete.\textsuperscript{39,41,49} By comparison, a previous pharmacokinetic study reported bioavailability ranging from 26 to 65\% after a single PO dose of furosemide when compared to a higher bioavailability of 96\% after PO administration of torsemide.\textsuperscript{8} A recent pharmacokinetic and pharmacodynamic study of orally-administered furosemide at 1 mg/kg in healthy horses showed extremely low bioavailability (approximately 5\%), absorption was erratic, and diuresis was not induced.\textsuperscript{53} It is possible that the furosemide dose used in the study was subclinical, and perhaps therapeutic plasma concentrations and clinical diuresis would have been observed if higher doses had been
used. Although torsemide was considered 5-10 times more potent than furosemide on a weight basis, we elected to use a considerably high dose (6 mg/kg) to increase the chances of absorption after a single PO administration during phase I.\textsuperscript{54} Based on drug accumulation theory and the pharmacokinetic data obtained in our study, it was speculated that PO dose of 2 mg/kg q12h would result in clinically meaningful drug concentrations in plasma. Therefore, this dosage regimen was applied for the pharmacodynamic study (Phase II).

In a pharmacodynamic study of torsemide in neonatal rabbits, a significant decrease in body weight was observed and related to the forced diuresis.\textsuperscript{50} An accurate measurement of feed intake was not obtained, but subjectively it was observed that all horses maintained their dry matter intake. This observation could indicate that torsemide had good palatability among the horses, but our data did not allow a definitive conclusion in this regard. Loop diuretics activate RAAS by decreasing intravascular volume.\textsuperscript{25} Aldosterone is an important regulator of renal reabsorption of sodium and effects blood pressure and volume.\textsuperscript{27} The aldosterone concentrations measured in our study indicated that orally administered torsemide activated the RAAS as shown by an increase in plasma aldosterone concentration, proving that orally-administered torsemide was sufficiently absorbed and induced significant diuresis, likely with a subsequent decrease in plasma volume.

Drugs that inhibit the action of aldosterone on the distal renal tubule potentially can decrease potassium loss during diuretic therapy. Torsemide has been shown to have an inhibitory effect on
aldosterone secretion by adrenal cells in rats, guinea pigs and cows. It was hypothesized that aldosterone secretion was decreased by torsemide, and decreased potassium excretion would be expected in response to the drug. In a study comparing the diuretic effects of torsemide and furosemide in dogs and cats, less potassium wastage was observed in the animals treated with torsemide. Also, in a study evaluating in vivo aldosterone receptor binding activity after administration of furosemide and torsemide to rats, the torsemide group experienced inhibition of aldosterone binding to its receptor in the cytoplasmic fraction of the kidney, whereas the furosemide group did not.

In our study, prolonged PO torsemide at 2 mg/kg q12 h induced a significant decrease in serum potassium concentration with moderate to severe hypokalemia noted in all horses during phase II. The marked decrease in serum potassium concentration required supplementation in all horses. After PO potassium chloride supplementation (0.1 g/kg, PO q12h), the decrease in serum potassium concentration reached a plateau, indicating that the electrolyte imbalance observed after PO torsemide can be managed and controlled using PO electrolyte supplementation.

The excessive potassium wastage observed in our study could have been related to the dosage that was employed. The dosage used in our study was relatively high, and might not be appropriate for the treatment of horses with CHF. The decision to use a high dosage was made with the intention of inducing marked diuresis, volume depletion and activation of the RAAS. In a previous
study performed with dogs and cats, torsemide at approximately 1/10 of the dose of furosemide induced longer diuretic effects. We believe that a reasonable dosage of torsemide for horses with CHF should be lower than used in our study. Based on clinical experience (data not published), we recommend a dosage of 0.5-1 mg/kg PO q12h, but further clinical studies are necessary to determine the optimal PO dosage of torsemide for the treatment of fluid retention in horses. The metabolic alkalosis noted in all horses, likely as compensation from the hypochloremia, also could have contributed to the marked hypokalemia observed in our study. Alkalosis has a direct influence on excretion of potassium mainly by affecting the Na+/K+-ATPase pump in the cortical collecting ducts.57

The effects of loop diuretics on systemic blood pressure and volume are of interest in the management of diseases such as systemic hypertension, exercise-induced pulmonary hemorrhage (EIPH) and CHF in the horse. Loop diuretics can to attenuate increased right atrial, venous, pulmonary arterial and capillary pressures, which are evident in the development of pulmonary capillary stress failure during exercise.58 Although the mechanism of EIPH is not yet fully understood, loop diuretics such as furosemide have been used for more than 40 years to decrease the occurrence or severity of EIPH in horses.53 Although a decrease in MAP was observed, which could be beneficial in the therapy of EIPH, further research with this new potent diuretic agent in EIPH is necessary before clinical use can be recommended. In our study, prolonged PO administration of torsemide induced a significant decrease in left atrial maximal diameter when
comparing baseline to day 6. Although a direct measure such as lithium dilution technique was not used to obtain stroke volume measurements in our study, non-invasive methods to determine stroke volume in horses using echocardiography have been described and provided reliable results.\textsuperscript{45} The decrease in the left atrial diameter likely was related to the hypovolemia induced.

In an open-label study performed in human patients with CHF secondary to left ventricular systolic dysfunction, patients assigned to treatment with torsemide were less likely to be hospitalized for CHF, were less fatigued, and reported better quality of life when compared to patients being treated with furosemide.\textsuperscript{59} In a study evaluating the effects of different diuretics on cardiac function in rats with induced CHF, animals being treated with torsemide showed decreased cardiac aldosterone synthase, less remodeling of the left ventricle and echocardiographic evidence of improved cardiac function when compared to rats being treated with furosemide.\textsuperscript{60} In dogs with CHF being treated with torsemide for 28 days, no evidence of weakness, fatigue, hypotension, severe electrocardiographic alterations or skin rash were observed.\textsuperscript{61} With the current availability of effective formulations of other cardiovascular medications, such as ACE inhibitors, for PO use, and the tendency to keep horses long term as companion animals, horse owners maybe more amenable to treat chronic medical conditions such as CHF.\textsuperscript{62} A diuretic drug such as torsemide, which potentially can provide better quality of life, less risk of cardiac remodeling, less diuretic resistance, and better owner compliance without the need for an injectable therapy regimen, can be an important addition in the therapy of CHF in the horse.
Conclusion

The use of orally-administered torsemide has several pharmacological advantages. Torsemide is relatively inexpensive and available in a wide range of tablet sizes (from 5 to 100 mg per tablet). Some of the electrolyte imbalances observed in our study were likely related to the high dosage used. As with other diuretics, horses being treated with torsemide should be closely monitored for any signs of dehydration and electrolytes imbalances. Based on the pharmacokinetic data and the pharmacodynamic effects observed, we recommend 0.5-1 mg/kg PO q12h as a starting dosage and recommend titrating the dosage to effect for horses with fluid overload. Good absorption after PO administration, a reasonable excretion half-life, and persistent diuresis may make PO torsemide an attractive alternative to furosemide for prolonged diuretic therapy in the horse.
References


Appendices

Appendix 1 - Individual mean plasma concentrations of torsemide (6mg/kg) over time following a single intragastric administration to 6 healthy adult mares.