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EFFECTS OF A SYNTHETIC SERINE PROTEASE INHIBITOR, CAMOSTAT MESILATE (FOY-305), ON MARKERS OF PANCREATIC ACINAR CELL DAMAGE, INFLAMMATION, AND FIBROSIS IN DOGS WITH SUSPECTED NATURALLY OCCURING CHRONIC PANCREATITIS

INAUGURAL-DISSERTATION
ZUR ERLANGUNG DES GRADES EINES
DOKTORS DER VETERINÄRMEDIZIN
AN DER FREIEN UNIVERSITÄT BERLIN

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ABBREVIATIONS used in this paper

AP	acute pancreatitis
BW	body weight
CBC	complete blood count
CM	camostat mesilate
CP	chronic pancreatitis
cPLI	canine pancreatic lipase immunoreactivity
CRP	C-reactive protein
cTLI	canine trypsin-like immunoreactivity
EPI	exocrine pancreas insufficiency
GI	gastrointestinal
HCT	hematocrit
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
QOL	quality of life
RI	reference interval
Spec cPL®	canine pancreas-specific lipase
TGF-β1	transforming growth factor β1

1 INTRODUCTION

Canine chronic pancreatitis is a continuing inflammatory condition of the pancreas that is characterized by irreversible histologic changes and often accompanied by the loss of function (Etemad et al., 2001; Bostrom et al., 2013). Fibrosis, lymphocytic inflammation and atrophy in various grades and stages are the most common findings during histopathology (Watson, 2012; Bostrom et al., 2013) and it is the permanence of morphological changes, rather than temporal factors that separates the chronic from the acute form (Watson, 2012). During late and end stages of the disease and only after gross depletion of reserve capacities, the progressive loss of parenchyma may result in exocrine and endocrine deficiencies, i.e. exocrine pancreas insufficiency and diabetes mellitus (Alejandro et al., 1988; Watson, 2003; Watson et al., 2010). Environmental (Steiner et al., 2008; Xenoulis et al., 2010) and hereditary (Bishop et al., 2010) risk factors are known contributors to the etiology of canine chronic pancreatitis. However, for the majority of cases there is an absence of precipitating risk factors and they remain idiopathic with the underlying cause unknown (Steiner, 2010). The pathogenetic mechanisms leading to chronic pancreatitis are complex and not completely understood, but extrapolated human and experimental data imply important roles for specialized fibroblasts (Shek et al., 2002; Apte et al., 2004) and cytokines (Shek et al., 2002; Apte et al., 2005). Recent publications suggest chronic pancreatitis is far more common among dogs than previously estimated (Watson et al., 2007; Watson et al., 2011). But because efficacious pharmaceutical agents are unavailable, treatment of these cases is limited to supportive care and management of complications. However, there are reports from Japan, where the protease inhibitor camostat mesilate (FOY-305) has been used for the treatment of chronic pancreatitis (Fujiwara, 1980; Hirayama, 1980; Horiguchi, 1980; Ishii, 1980; Ishii, 1984) in humans. There are no studies about the efficacy of camostat mesilate for the treatment of chronic pancreatitis in dogs. Yet the current literature suggests that it might directly influence pathophysiologic mechanisms of the disease via inhibition of lytic enzyme cascades (Tamura et al., 1977; Sakaguchi, 1980; Bonner et al., 1987), cytokines and pancreatic stellate cells (Sugiyama et al., 1996; Su et al., 2001; Gibo et al., 2005; Jia et al., 2005). Therefore, the goals of this study were to investigate the efficacy of camostat mesilate in dogs with suspected chronic pancreatitis and quantify its impact on acinar cell damage, as well as markers of fibrosis and inflammation.

2 LITERATURE REVIEW

2.1 The pancreas

2.1.1 History

Discovery of the pancreas is largely credited to Greek anatomist and surgeon Hirophilus of Alexandria around 300 B.C. (Wiltse et al., 1998). It was Ruphos of Ephesos who, roughly 400 years later, took into account the fleshy structure of the organ and named it. At the end of the second century A.D., Galen spuriously described the pancreas as a cushion for the stomach - a statement set in stone for thirteen hundred years. Until the grip of the Roman Catholic Church on science thawed and anatomists and physicians of the renaissance began to investigate the human body.

In 1561, Gabriele Falloppio remarked that, due to the anatomical location of the pancreas, no animal walking on all fours would benefit from its cushioning protection. It was in 1642 that Wirsung discovered the pancreatic duct (Beger et al., 2009). Sylvenius and de Graaf of Leyden postulated two decades later that pancreatic juices aided the segregation of food items. By the end of the 17th century, enough of the pancreas' function was known for Sommering to refer to it as an abdominal salivary gland, or *Bauchspeicheldruese* – still the common term in German speaking countries. With the discovery of the sphincter of Oddi new anatomic findings became rare and science turned to histologic and physiologic details. When Ivan Pavlov received the Nobel Prize in Medicine for his work on digestive physiology in 1904, Eberle, Bernard, Kuhne, and others had generally established the pancreas' role in digestion and elucidated the purpose of its enzymes (Eberle, 1834; Howard et al., 2002).

Diabetes mellitus, the oldest endocrine disease known to man, was first cured with pancreas extracts in a pancreatectomized dog in 1922 by Banting and Best. Only 6 months later, a 14 year-old boy became the first human patient successfully treated for the disease (Banting et al., 1922) - 22 centuries after Hippocrates, the paragon of antiquity's physicians, described it. The same year, Banting, Macleod, Best and Collip discovered insulin. Although two of the four were denied the deserved recognition, the Nobel Prize in Physiology or Medicine, which was awarded to Banting and Macleod in 1923 (Nobelprize.org, 2014) for the discovery of insulin bears testament to the impact and importance of their work nonetheless.

2.1.2 Embryology and anatomy

The canine pancreas is a slender, soft, pinkish-grey secretory organ bearing gross resemblance to the salivary gland (Dyce et al., 2010). In a 14 kg dog it measures on average 25 cm in length and weighs approximately 31 grams (Evans et al., 1993). The pancreas is a retroperitoneal organ and its corpus can be found directly adjacent to the duodenum, with a lobus pancreaticus dexter and lobus pancreaticus sinister extending to their respective sides (Nickel et al., 1979). According to Noden *(Noden et al., 1985),* the pancreas is formed from two separate endodermal extensions of the foregut – the lobus pancreatis sinister is derived from the dorsal pancreatic diverticulum, while the lobus pancreatis dexter is derived from the ventral pancreatic diverticulum and the corpus is formed when the two lobes cross and fuse**.** Each diverticulum also forms a pancreatic duct, both of which communicate and usually persist in the dog (Noden et al., 1985). The lesser, accessory pancreatic duct originates from the dorsal diverticulum and empties into the duodenum on the minor papilla. From the ventral primordium comes the greater pancreatic duct. Although smaller in size, it empties on the major papilla along with the common bile duct (Nickel et al., 1979; Dyce et al., 2010). Smooth sphincter muscles seal the pancreatic ducts from the duodenal lumen, prevent regurgitation of duodenal fluids, and ensure a unidirectional flow of pancreatic juice and bile (Kyösola et al., 1974).

2.1.3 Histology

The pancreas has a finely lobulated architecture, and is covered by a collagenous capsule (Young et al., 2014). Fine fibrous membranes divide and connect the lobules, which are visible on gross examination with the naked eye (Burdick, 2006). Blood and lymphatic vessels, as well as interlobular ducts, which are part of a highly branched duct system (Young et al., 2014) that serves to empty the alkaline and enzyme-rich excretions of the exocrine pancreas into the duodenum are located within the septa.

The functional cells of the exocrine pancreas are the acinar cells (**Figure 1**). They are pyramid shaped and have one, sometimes two round nuclei that are surrounded by basophilic cytoplasm. Due to their extensive secretory function, acinar cells also possess a prominent Golgi-apparatus. Thus, on electron micrographs, dark, electron-dense acidophilic zymogen granules can be seen in the apical portion of each acinar cell (Burdick, 2006). Berry-like groups of acinar cells constitute an acinus, the smallest functional unit of the exocrine pancreas. Acini make up roughly 80% of the organ mass (Burdick, 2006) and secrete into blind-ending intercalated ducts, lined by specialized bicarbonate-secreting centroacinar cells (Samuelson, 2007). The intercalated ducts, in turn, empty into the increasingly larger intra- and interlobular ducts (Young et al., 2014). Reflux of secretions into the intercellular space is prevented by tight junctions between the apical ends of acinar cells (Metz et al., 1977; Lodish, 2000).

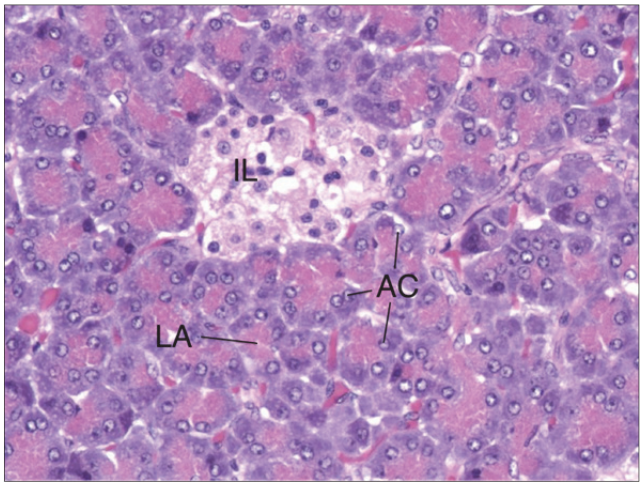

Figure 1 Example of normal canine pancreas tissues. HE, acinar cells (AC), islet of Langerhans (IL), lumen of acini (LA). *Courtesy of Dr. K. Burke*

Scattered throughout the pancreas and making up 2% of the gross organ mass (Burdick, 2006) are light-staining clusters of neuroendocrine cells called islets of Langerhans (Langerhans et al., 1869; Young et al., 2014). Each is usually composed of 20 to 30 cells, but roughly one tenth of the cells can be found at extrainsular sites, predominantly in ductular epithelia and connective tissue (Hawkins et al., 1987; Wieczorek et al., 1998). In the dog, Langerhans islets are host to α, β, δ and PP cells that engage in the synthesis and release of a variety of regulatory neuroendocrine peptides. There are marked interspecies differences in islet microstructure, as well as local distribution of islet-forming cells (Hawkins et al., 1987; Redecker et al., 1992; Wieczorek et al., 1998; Steiner et al., 2010). In canine pancreata, single glucagon secreting α-cells and insulin-secreting β-cells can occasionally be found scattered among exocrine tissue of the right pancreas lobe, whereas in the left lobe, cell conglomerates located in the center or periphery of the islets of Langerhans dominate (Wieczorek et al., 1998).

2.1.4 Physiology

2.1.4.1 Exocrine

Among others, the primary function of the exocrine pancreas is to facilitate digestion of macronutrients in the intestinal lumen, through the production of pancreatic enzymes and pro-zymogens. In doing so, it surpasses all other organs in quantity of daily protein synthesis (Gorelick, 2006; Logsdon et al., 2013). Proteolytic, amylolytic, lipolytic, and nucleolytic enzymes and precursors of such enzymes are synthesized through protein synthesis, where DNA is transcribed to rRNA, which in turn is translated into a polypeptide strand by ribosomes (Gorelick, 2006). The polypeptide then moves through the extensive rough endoplasmatic reticulum (Jamieson et al., 1971), where removal of a signal peptide by signal peptidase (Martoglio et al., 2003), and protein folding results in enzymes and pro-enzymes (Steiner, 2008). Secretory proteins undergo substantial enrichment (Oprins et al., 2001) and are subsequently translocated to the Golgi complex via direct tubular connections (Case, 1978) or vesicles. There, the nascent proteins undergo glycosylation and other protein modifications. Active digestive enzymes and pro-enzymes are stored in zymogen granules, which fuse with the apical plasma membrane of acinar cells in response to secretagogues (Wasle et al., 2002), leading to exocytosis and secretion into the lumen of the acini.

While acinar cells secrete enzymes and zymogens, cells forming the intercalating, intralobular and interlobular ducts are tasked with the neutralization of gastric acid within the small intestine. Upon stimulation by secretin from enterochromaffine cells, they secrete large amounts of bicarbonate-rich fluid (Fölsch et al., 1977), the volume of which surpasses the total volume of all other fluids released by acinar cells (Ross et al., 2006). In addition to water and bicarbonate, pancreatic juice contains sodium, potassium, and chloride ions, making it isotonic with plasma. Flow rates of ductal secretions vary according to each particular digestive phase. They are increased after meals, when secretin stimulates secretion and revert to a low baseline flow rate during interdigestive states. Neither potassium nor sodium levels of ductular secretions vary with a changing flow rate. However, at high flow rates an increase in bicarbonate concentration, and a simultaneous decrease in chloride concentration can be observed (Pandol, 2010).

Table 1: Products of the exocrine pancreas

Active Enzymes		Zymogens		Others	
	Function		**Function**		**Function**
Amylase	*Hydrolysis of dietary starch*	Chymotrypsinogen	*Proteolysis*	Bicarbonate	*Neutralization of gastric acid*
Carboxylesterase	*Hydrolysation of ester-bonds*	Procarboxypeptidase	*Proteolysis*	Intrinsic factor	*Cobalamin absoption*
DNAse	*Hydrolysis of phosphodiester bonds in DNA strands*	Proelastase	*Proteolysis*	Pancreatic Secretory Trypsin Inhibitor	*Blockade of trypsin activity*
Lipase	*Hydrolysis of triglycerides to monoglycerides and fatty acids*	Prophospholipase	*Hydrolysis of phospholipids*	Procolipase	*Prevents inhibition of lipase by bile-salts*
RNAse	*Hydrolysis of phosphodiester bonds in RNA strands*	Trypsinogen	*Proteolysis*	Water, ions	*Hydration of secretions*

It lies in the nature of some pancreatic enzymes to digest components of cellular membranes. Hence these enzymes pose a constant threat of autodigestion to the pancreas (Steiner, 2008). Among them are trypsin, chymotrypsin, elastase, and phospholipase. In order to prevent autodigestion, a number of safety mechanisms are in place. Firstly, potentially dangerous enzymes are not secreted in their active and functional state, but rather enter the duodenal lumen as inactive pro-enzymes or zymogens (Case, 1978; Pandol, 2006). Conversely, enzymes that find their substrate within the cell can be safely secreted in the active form (Pandol, 2006). Secondly, because lysosomal contents are capable of intracellular trypsinogen activation (Figarella et al., 1988; Gaisano et al., 2009), storage of zymogen granules and lysosomes is strictly separated. Despite an environment within the zymogen granule that is prohibitive to autoactivation, small quantities of trypsinogen are constantly converted to trypsin even under physiologic conditions (Steiner, 2008; Nathan et al., 2010). Therefore, and thirdly, acinar cells synthesize pancreatic secretory trypsin inhibitor (PSTI), which is stored in zymogen granules (Fukuoka et al., 1986) alongside enzymes and zymogens, and irreversibly binds and inactivates prematurely activated trypsin (Pubols et al., 1974; Greene et al., 1976; Bartelt et al., 1977).

Apart from its role in proteolysis, trypsin engages in a pivotal role in the activation of all other zymogens (Rinderknecht, 1993). In order to activate zymogens within the small intestine, enteropeptidase from duodenal and jejunal enterocytes, discovered by Pavlov (Pavlov, 1910), is required to cleave a peptide from trypsinogen, thus converting it to trypsin (Hermon-Taylor et al., 1977; Pandol, 2006). This in turn triggers a pancreatic enzyme cascade that activates additional trypsinogen and eventually converts all other zymogens into their active form (Rinderknecht, 1993).

The major physiologic processes of exocrine pancreatic secretion in response to a meal can be divided into distinct phases (Rhoades et al., 2013), each of which are subject to neural and hormonal regulators and involve complex varying secretory and inhibitory inputs and regulatory signals (Liddle, 2006). During the *cephalic* phase, stimuli like the thought, smell or taste of food result in a mediator-dependent release of a predominantly enzyme-rich pancreatic juice. Interestingly, although studies have shown that electrical stimulation of vagal nerve fibers cause pancreatic secretion (Bourde et al., 1970; Kaminski et al., 1975; Holst et al., 1979), only 50 to 60% of pancreatic secretions in humans can be attributed to a meal-dependent response (Beglinger et al., 1985; Gullo et al., 1988). Vagal reflexes are not only responsible for secretion during the *cephalic* phase, but also mediate responses to gastric and intestinal stimuli (Liddle, 2006). The secretory response of the *gastric* phase is responsible for roughly 10% of the overall pancreatic response to a meal (Liddle, 2006) and relies on two triggers. Firstly, as demonstrated by balloon distention (White et al., 1960; Vagne et al., 1969), gastropancreatic reflexes such as the distention of the gastric wall. And secondly, products of gastric digestion such as peptides, fatty acids, and monoglycerides (Liddle, 2006). Complex interactions between macronutrients, micronutrients, gastric acid, bile acids, as well as neural and hormonal factors (Liddle, 2006) are involved in the regulation of the *intestinal* phase of pancreatic secretion. Up to 70% of pancreatic secretions may be released during this phase (Liddle, 2006), which facilitates the neutralization of chyme and the decomposition of meal components into absorbable particles.

Gastric pH in healthy dogs is approximately 1.0 (Sagawa et al., 2009). However, the optimal pH for pancreatic enzymes is in the alkaline pH range. Studies have demonstrated that pH in the lumen of the canine duodenum abruptly increases from 2.0 to 5.5 within the first few centimeters (Brooks et al., 1970; Rune, 1973). This led to the discovery that gastric acid is the driving force behind pancreatic bicarbonate secretion, which is responsible for this pH increase (Liddle, 2006). The release of bicarbonate is mediated through the release of secretin from duodenal enteroendocrine APUD cells (Polak et al., 1971; Liddle, 2006). Secretin is a potent stimulant of pancreatic fluid and bicarbonate secretion (Chey et al., 2003; Steward et al., 2005; Chey et al., 2014). Additional hormones and neurotransmitters, notably cholecystokinin and acetylcholine, act as synergists during this process (Chen et al., 2001; Liddle, 2006). Interestingly, the magnitude of pancreatic bicarbonate secretion is not only proportional to the amount of gastric acid entering the duodenum, but also to the section length of stimulated small intestine (Solomon et al., 1978; Fink et al., 1982).

The impact of protein and its digests on pancreatic secretion has been well studied in dogs and it has been established that some protein digests elicit a viable secretory response (Meyer et al., 1976; Meyer et al., 1976) through vagal reflexes and cholecystokinin (Beglinger et al., 1984; Murphy et al., 2006). Cholecystokinin (CCK) is a neuropeptide that originates from duodenal and jejunal I-cells (Larsson et al., 1978) and acts as a stimulating mediator for digest-induced pancreatic enzyme secretion. Especially tryptophane and phenylalanine exhibit a high activity for CCK release (Konturek et al., 1973). While a number of amino acids act as stimulants (Wang et al., 1951; Wolfe et al., 1975; Meyer et al., 1976), it appears that peptides exert a stronger stimulus on the exocrine pancreas (Meyer et al., 1974; Meyer et al., 1976) than intact proteins, which trigger a significantly weaker or no response at all.

Secretion caused by fatty acids depends on the length of their aliphatic tail. In dogs, 8 or more carbon atoms are necessary to lead to significant secretion (McLaughlin et al., 1999), whereas intact triglycerides fail to stimulate it (Meyer et al., 1974). Interestingly, the importance of the C-chain length appears reversed in bicarbonate secretion, where short chain fatty acids are the significantly stronger stimulus (Liddle, 2006).

The effects of CCK are countered by somatostatin, which acts in a paracrine fashion on adjacent cells but is also released into the circulation post-prandially. Somatostatin not only inhibits the release of bicarbonate (Hanssen et al., 1977; Konturek et al., 1981; Konturek et al., 1985; Hildebrand et al., 1992), but also leads to a decrease in enzyme secretion through inhibition of pro-secretory hormones and neurotransmitters (Liddle, 2006).

Dietary cobalamin (vitamin B12) is absorbed by ileal brush-border enzymes but its absorption requires intrinsic factor as a mediator. In contrast to humans, where this glycoprotein is synthesized predominantly by gastric parietal cells (Festen, 1991), canine intrinsic factor originates to a great extent from pancreatic duct cells (Simpson et al., 1993).

2.1.4.2 Endocrine

The endocrine pancreas consists of 4 major cell types, which secrete the endocrine and paracrine hormones insulin, glucagon, pancreatic polypeptide, and somatostatin. Pancreatic hormones are involved in the regulation of blood glucose levels but their effects on each other are sometimes not fully understood. In their entirety, they are part of a complex and dynamic system that ensures normoglycemia.

Insulin is a peptide hormone that originates from β-cells in the islands of Langerhans. Because internalization of glucose relies on insulin-dependent receptors, it is the only hormone capable of lowering blood glucose concentrations. The release of insulin is primarily and directly triggered by elevated post-prandial blood-glucose concentrations, which leads to increased uptake of glucose into β-cells by means of dedicated GLU transporters (Lieberman et al., 2013), causing calcium-dependent intracellular events that culminate in the exocytosis of secretory vesicles and subsequent release of insulin. Since insulin is an anabolic hormone, it also up-regulates DNA replication and protein synthesis. Conversely, glucagon is the principal counter-regulatory hormone opposing the anabolic effects of insulin (Unger, 1985). Circulating insulin decreases under hypoglycemic conditions.

Table 2: Products of the endocrine pancreas

Cell type	Secretion	Function
α-cells	Glucagon	*Increases blood glucose levels*
β-cells	Insulin	*Regulation of carbohydrate and fat metabolism, lowers blood glucose levels*
δ-cells	Somatostatin	*Inhibition of insulin, glucagon, growth hormone and others*

Glucagon is a 29 amino acid hormone synthesized in α-cells by cleavage of the larger preproglucagon and further post-translational processing within the endoplasmatic reticulum (Lieberman et al., 2013). Glucagon stimulates hepatic glycogenolysis, gluconeogenesis, and ketogenesis. Low levels of blood glucose and amino acids are the major stimulus of glucagon release, whereas high blood glucose concentrations (Gylfe et al., 2014) and, indirectly, insulin (Ishihara et al., 2003) are generally believed to suppress its release. In the mouse and rat pancreas, α-cells are also stimulated in an autocrine fashion by the binding of glucagon to specialized receptors (Ma et al., 2005). However, significant controversy still surrounds the relationship between insulin and glucagon release under hyperglycemic conditions (Vieira et al., 2007; Gylfe et al., 2014).

While insulin and glucagon are directly involved in glucose homeostasis, somatostatin and pancreatic polypeptide exert finer regulatory functions. Somatostatin, synthesized by δ-cells, is a paracrine, inhibitory hormone with effects on glucagon and insulin homeostasis

(Hauge-Evans et al., 2009; Hauge-Evans et al., 2010). Somatostatin has a suppressive effect on both hormones (Starke et al., 1987; Hildebrand et al., 1991) and mediates glucagon inhibition by hyperglycemia (Klaff et al., 1987). Pancreatic polypeptide released by PP cells is considered the counterpart to pro-secretory CCK. It acts as an inhibitor of the endocrine, as well as exocrine parts of the pancreas (Taylor et al., 1979; Murphy et al., 1981; Konturek et al., 1982) and has shown to increase after a meal or a number of experimental stimuli such as duodenal acidification or perfusion with amino acids (Beglinger et al., 1984; Beglinger et al., 1984).

2.2 Chronic pancreatitis

There appears to be great inconsistency regarding the differences between acute and chronic pancreatitis in dogs in the literature. Quite often, authors only refer to pancreatitis in general and fail to differentiate between the two forms. This is especially true for older veterinary papers, but less so for newer publications from this field. In the past, this practice may have been precipitated by the virtually indistinguishable clinical appearance of both entities, the lack of a universally applicable classification system, and available diagnostic methods. Veterinary medicine has since benefitted from vastly advanced diagnostic modalities. Imaging equipment and techniques have grown in quality and availability; old biomarkers have been abandoned for more specific ones. These advances have translated into enhanced diagnostic potential. Differentiation between the various forms of the disease has raised awareness for their dissimilarities and sparked investigations into their etiology and pathophysiology.

2.2.1 Prevalence

Recent studies basing prevalence of CP on histopathological confirmation found evidence suggestive of CP in an unexpectedly large number of dogs. Newman reports that pancreata from 47 out of 73 (64%) dogs presented for necropsy showed histological evidence of suppurative inflammation, necrosis, or lymphocytic infiltration, and 28 (38.4 %) showed either of these signs in conjunction with fibrosis (Newman et al., 2004). Watson et al. evaluated 200 pancreata from unselected dogs submitted for necropsy for signs of chronic pancreatitis and reported a prevalence of 34% when omitting autolyzed cases (Watson et al., 2007). This suggests CP is an often overlooked and clinically underdiagnosed, but significant disease in dogs (Watson et al., 2010; Watson et al., 2011; Watson, 2012). However, it is unclear to what extent these findings translate into clinical disease. Interestingly, histopathologic evaluation of human pancreata indicates that, even in patients without any clinical signs, CP may be more common than previously estimated (Pitchumoni et al., 1984).

2.2.2 Etiology

The TIGAR-O (**T**oxic-metabolic, **I**diopathic, **G**enetic, **A**utoimmune, **R**ecurrent severe acute pancreatitis-associated chronic pancreatitis and **O**bstructive chronic pancreatitis) classification system by Etemad and Whitcomb outlines etiologies associated with the development of chronic pancreatitis in people (Etemad et al., 2001), according to approximate prevalence. In lieu of a comparable system for veterinary medicine, this classification is frequently extended to summarize the etiologies of canine CP. Often times, singular environmental and genetic risk factors in this system are linked in complex relationships whose exact nature eludes us.

For example, the premiere risk factor of CP in people in the industrialized world is alcoholism (toxic-metabolic) and precedes disease in 55 to 88%. Yet long-term high-dose alcohol feeding does not necessarily lead to CP in animals and only 10% of severe alcoholics develop disease (Tyler at al., 2004) . This suggests that the toxic-metabolic component is a cofactor that requires a predisposing genetic component in order to lead to chronic pancreatitis. As some of these relationships unravel, similar connections between risk factors are made in dogs, where Miniature Schnauzers, Cavalier King Charles Spaniels, Cocker Spaniels, and Boxers present more often with CP than do dogs of other breeds (Watson et al., 2007; Watson et al., 2011). Indeed, not only do disproportionately large numbers of Miniature Schnauzers have hypertriglyceridemia (Xenoulis et al., 2007), which in itself is a risk factor for AP or CP in humans and dogs (Cameron et al., 1973; Hess et al., 1999; Hess et al., 2000; Yadav et al., 2003; Xenoulis et al., 2010; Xenoulis et al., 2010) but not an uncommon finding in the general dog population (Comazzi et al., 2004). But also does this finding positively correlate with elevated cPLI concentrations (Xenoulis et al., 2010). Yet hypertriglyceridemia in this breed does not automatically translate into disease (Xenoulis et al., 2011), which suggests the existence of a genetic cofactor or modifier.

Drugs as toxic-metabolic risk factors play no documented role in the etiology of canine CP. However, elevated cPLI concentrations have been connected to treatment with the anticonvulsants phenobarbital and potassium-bromide (Steiner et al., 2008) and pancreatitis may be a severe adverse effect of these medications. Because both drugs are used as maintenance anticonvulsants, this increase in PLI concentrations indicative of acinar cell damage could potentially persist for long periods of time. However, there are no studies that investigate potential correlations between treatment with these medications, cPLI concentration and histopathologic signs of CP.

Most cases of canine chronic pancreatitis are considered idiopathic because the underlying cause cannot be established. In addition to research of the complex underlying genetic architecture involving multiple loci, discoveries of novel toxic, environmental and metabolic risk factors have diminished this category in human medicine (Etemad et al., 2001; Masson et al., 2013). A cationic-trypsinogen mutation that inhibits trypsin auto-deactivation (Whitcomb et al., 1996) and loss-of-function mutations in genes encoding for pancreatic secretory trypsin inhibitor (SPINK1; PSTI) (Witt et al., 2000) are examples thereof. These findings lie at the molecular basis of repeated acute and chronic pancreatitis or act as disease modifiers (Pfutzer et al., 2000). There are few genetic studies in dogs but mutations of the gene encoding for PSTI have also been discovered in Miniature Schnauzers (Bishop et al., 2010). The functional correlates of these findings remain to be elucidated.

2.2.3 Pathophysiology

The currently accepted hypothesis for the pathophysiology of pancreatitis in dogs is largely derived from the extrapolation of data from human and experimental murine models. It is unclear if this accurately reflects the mechanisms of naturally occurring pancreatitis in the dog at all times (Su et al., 2006) and underlying processes of the disease are complex and often remain poorly understood. The hypothesized basis of CP is the repetition of acute pancreatic tissue damage, which results from sequential events that begin with premature intracellular zymogen activation and culminate in autodigestion (Steiner, 2010). The only exceptions to this rule are the rare cases of infectious pancreatitis, where blood-borne microorganisms directly cause cell damage.

A widely accepted idea that best explains the initiating steps in acute pancreatitis is the co-localization theory (Mansfield, 2012). First, secretory blockage of acinar cells leads to the co-localization of zymogen granules and lysosomes (Rinderknecht, 1986). Normally, both are physically strictly separated while stored in the apical portion of acinar cells (Scheele, 1980). As a result, zymogen granules and lysosomes co-localize and fuse to form giant vacuoles (Simpson, 1993). There is mounting evidence that suggests alterations in intracellular pH (Sherwood et al., 2007; Bhoomagoud et al., 2009), and changes in intracellular Ca2+ homeostasis (Gerasimenko et al., 2014) have an important effect on this event. Exposure to lysosomal proteases, foremost cathepsin B (Rinderknecht, 1986), and to low intra-vacuolar pH (Niederau et al., 1988) are key events that subsequently result in the activation of trypsinogen to trypsin. Only up to 10% of the auto-activated intracellular trypsin

can be neutralized by pancreatic secretory trypsin inhibitor (Laskowski et al., 1980; Rinderknecht, 1986). Once this threshold is overcome, trypsin precipitates the activation of more trypsinogen and, in consequence, other zymogens. Lipase, trypsin, and phospholipase A2 cause direct damage to cellular components (Williams et al., 1996) and the ensuing cell damage leads to necrosis, localized inflammation, leukocyte migration and release of cytokines (**Figure 2**) (Steiner, 2010; Mansfield, 2012; Mansfield, 2012). In fact, some enzymes and zymogens, foremost trypsin, are capable of directly attracting neutrophils (Keck et al., 2005).

Reactive oxygen species and cytokines secreted by neutrophils advance localized inflammation and attract more inflammatory cells (Frossard et al., 1999). During this process, affected tissues become necrotic and the microcirculation is negatively impacted (Cuthbertson et al., 2006). This results in the interdependent release of additional acinar cell contents, including enzymes and zymogens. Further autolysis, recruitment of neutrophils, and exacerbation of inflammation are the consequence (Cuthbertson et al., 2006).

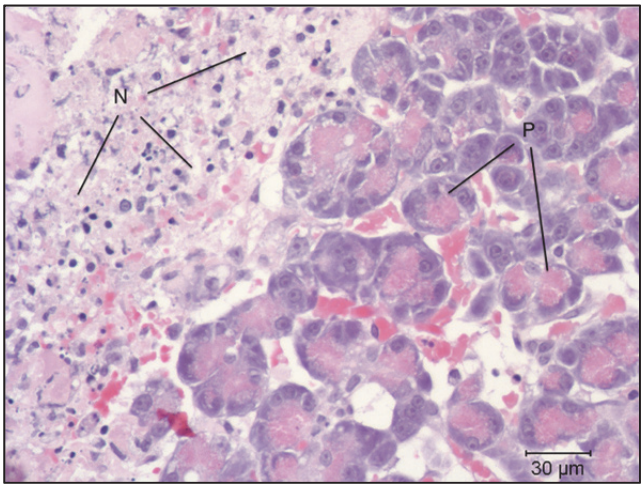

Figure 2 Pancreatic necrosis in a dog. Necrotic (N) areas and normal pancreatic tissue (P) can be observed. H&E stain, magnification 40X *Courtesy of Dr. K. Burke*

The model that best explains the progression from acute to chronic is the necrosis-fibrosis hypothesis. It stipulates that necroinflammation and mounting residual damage from recurrent attacks of acute pancreatitis result in irreversible tissue damage, fibrosis, and atrophy (Kloppel et al., 1993; Witt et al., 2007). In humans, this series of inflammatory events involves interstitial fat necrosis, distortion and stenosis of interlobular ducts, intra-ductular protein precipitation, and subsequent blockage of pancreatic secretion. In turn, this exacerbates inflammation and causes the disappearance of upstream acinar tissue (Kloppel et al., 1992).

Progression of AP to CP in alcoholics correlates with the number and severity of acute attacks (Ammann et al., 1994; Mullhaupt et al., 2005) and a comparable evolution may be assumed for dogs. However, it should be noted that the necrosis-fibrosis hypothesis is also

applicable to non-alcohol related pancreatitis in humans (Witt et al., 2007). Recurrent necroinflammatory events that lead to fibrosis in CP have been reproduced in animal models of CP as well (Puig-Divi et al., 1996; Haber et al., 1999). Research in this field has drawn attention to pancreatic stellate cells (PSC), which bear morphologic and functional similarities to the fibroblast-like stellate cells (Ito-cells) involved in hepatic fibrosis (Friedman, 1993; Benyon et al., 2001; Kocabayoglu et al., 2013). The role of quiescent PSCs is not entirely understood (Jaster, 2004), but activated PSCs appear to play an important part in pancreatic fibrosis by synthesizing and degrading extracellular matrix proteins (Apte et al., 2004). There are at least two known factors that lead to the activation of PSCs. In vivo, they are activated either directly by oxidants or reactive oxygen species - as is the case in humans, when alcohol and its metabolites cause direct cellular stress - or by cytokines such as tumor necrosis factor-alpha (TNF), transforming growth factor (TGF)-β, IL-6, and IL-1 (Kloppel et al., 1992; Apte et al., 1999; Apte et al., 2005). Once transitioned from their quiescent state, PSCs are capable of synthesizing and secreting cytokines (especially TGF-β) autonomously, resulting in perpetuation of the inflammatory process (Shek et al., 2002; Sparmann et al., 2005). TGF-β1 is a multifunctional cytokine that controls cellular proliferation, as well as differentiation. Its interaction with fibroblastic cells is an integral part of various physiologic processes, for example the wound healing process. However, TGF-β1 also plays a pivotal role in fibrosis in the pancreas and other tissues (Van Laethem et al., 1995; Branton et al., 1999; Okuno et al., 2001). It is up-regulated along with its precursors secondary to pancreatic inflammation in humans (Van Laethem et al., 1995) and participates in chronic pancreatitis via increased deposition of extra cellular matrix proteins in transgenic mice that overexpress it (Sanvito et al., 1995; Vogelmann et al., 2001).

Although our current knowledge about PSCs and TGF-β1 is largely based on experimental *in vitro* and *in vivo* models, the growing understanding of their role in the perpetuation and potentiation of pancreatic fibrosis makes it seem plausible that similar pathways and mechanisms are involved in canine chronic pancreatitis.

2.2.4 Diagnosis and clinical features

A diagnosis of chronic pancreatitis can be based on morphologic and histologic criteria, clinical and functional findings or a combination thereof (Etemad et al., 2001). However, diagnostic tools available to small animal clinicians differ greatly in utility and it is impossible to differentiate acute from chronic pancreatitis in dogs based on clinical signs alone. As a matter of fact, even dogs with histologic signs of acute pancreatitis, which causes more severe signs, may be clinically unremarkable (Ruben et al., 2009). It is therefore considered impossible to conclusively diagnose chronic pancreatitis without histopathologic confirmation.

A recent retrospective study identified decreased appetite (98%), lethargy (93%) and vomiting (88%) as the most common clinical features among 40 dogs with histopathological confirmation of CP. Diarrhea and abdominal pain were reported in 49% and 35% of dogs, respectively (Bostrom et al., 2013). These signs may be low-grade and intermittent (Watson, 2012) and are preceded by a history of vomiting in approximately half of all cases (Bostrom et al., 2013). Postprandial abdominal pain may result in learned food aversions that resolve under analgesic treatment (Watson, 2012). Occasionally, dogs with a history of subclinical disease or mild symptoms display signs more commonly associated with acute illness (Watson, 2012). Other possible findings that can sometimes be linked to CP are endo- and exocrine deficiencies following extensive replacement of pancreas tissue. Although data investigating the linkage between persistent pancreatic inflammation and its consequences are sparse, one study found that 4 out of 11 (36%) dogs with exocrine pancreas insufficiency (EPI) had concurrent chronic pancreatitis (Watson, 2003), suggesting that CP may be the second most common cause of canine EPI. Similarly, EPI and subsequent maldigestion are well-established consequences of late, and end-stage CP in humans (Lankisch et al., 1993) and cats (Hoskins et al., 1982). Involvement of the endocrine pancreas in CP can result in the loss of β-cells and potentially, subsequent diabetes mellitus. One study found histological confirmation of CP in 5 out of 18 (28%) diabetic dogs (Alejandro et al., 1988) .

Because no finding is specific for chronic pancreatitis in dogs, routine laboratory testing, such as complete blood count (CBC), urinalysis, and serum chemistry profile are of little diagnostic value (Steiner, 2010). Yet general clinical pathology remains valuable for the assessment of a patient's overall health status and direction of further treatment.

Imaging techniques readily available to clinicians, i.e. ultrasonography and radiography, are of limited use for the diagnosis of chronic pancreatitis. Abdominal radiography suffers especially from a very low sensitivity (24%), even in fatal acute cases of pancreatitis (Hess et al., 1998), where more pronounced lesions and increased soft tissue opacity aids identification (Kealy et al., 2011). Basing diagnosis on changes in echogenicity (Hess et al., 1998; Kealy et al., 2011), transabdominal ultrasonography can be highly specific (up to 70%) (Hess et al., 1998) in acute cases. Yet it is very dependent on operator skill and lesions commonly seen with chronic pancreatitis are more subdued than acute ones. Also, the hyperechogenicity associated with CP is only rarely ultrasonographically observed (Steiner, 2010). There are no scientific papers that evaluate the sensitivity or specificity of transabdominal ultrasonography for the diagnosis of chronic pancreatitis in dogs to date.

There are no laboratory assays specifically for the diagnosis of CP and only two assays are currently used to evaluate pancreatic functional status and damage. The canine Trypsin-like immunoreactivity (cTLI) assay is based on the quantification of trypsinogen and trypsin that is released into vascular space (Steiner, 2008; Steiner, 2014). The measurement of serum cTLI is a useful tool for diagnosis of exocrine pancreas insufficiency, which is associated with a drastic decrease of serum cTLI concentration (Batt, 1993). During pancreatic inflammation, serum cTLI concentrations may be increased in some individuals (Steiner, 2014), but the sensitivity is low and increased serum trypsin-like immunoreactivity concentrations were only seen in 17% of dogs with chronic pancreatitis in one study (Watson et al., 2010). Because EPI is seen in some cases of canine CP, cTLI may be a valuable diagnostic indicator for clinicians and prompt further investigation.

In contrast to traditional lipase activity assays, serum canine pancreatic lipase immunoreactivity (cPLI) is highly specific for lipase that originates from pancreatic acinar cells, enabling the targeted evaluation of possible acinar cell damage. cPLI is readily available and has been validated for canine serum (Huth et al., 2010). Based on a number of studies (Neilson-Carley et al., 2011; Trivedi et al., 2011; Haworth et al., 2014; Steiner, 2014), serum PLI concentration is the most sensitive and specific diagnostic test for pancreatitis currently available in dogs (Steiner, 2014). However, false-positive as well as false-negative results can be observed (Watson et al., 2010; Haworth et al., 2014) and PLI should be used in conjunction with a thorough clinical workup in order reach a conclusive diagnosis (Mansfield, 2013). It is important to recognize that cPLI results reflect on a condition (leakage of pancreatic lipase from acinar cells), which is most likely caused by inflammation

and, if persistent, may result in chronic pancreatitis. It does not, however, give any information about the nature and degree of histologic changes or how long this condition has persisted. If repeated cPLI measurements are used, any diagnosis of CP must be classified as "suspected".

Biopsies and subsequent histopathology used to be considered the gold standard for the diagnosis of pancreatitis and provide the highest diagnostic value (Spillmann et al., 2000). Although endoscopic fine needle aspiration under sonographic guidance is a feasible and safe procedure (Kook et al., 2012), a number of caveats may limit the usefulness of pancreatic biopsies in a clinical setting. Firstly, gross lesions may not be apparent (Newman et al., 2004) and secondly, the distribution of pancreatic lesions can be highly localized, such that pancreatitis cannot be safely ruled out even if several biopsy specimens are being collected

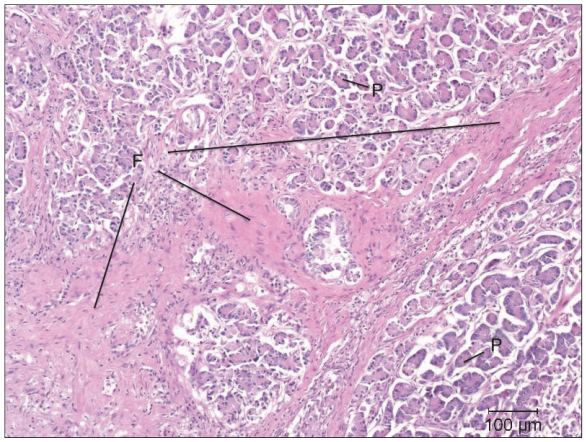

Figure 3 Pancreatic fibrosis in a dog. Large connected areas of fibrosis (i.e., bridging fibrosis) can be observed. H&E stain; magnification 40X, *Courtesy of Dr. K. Burke*

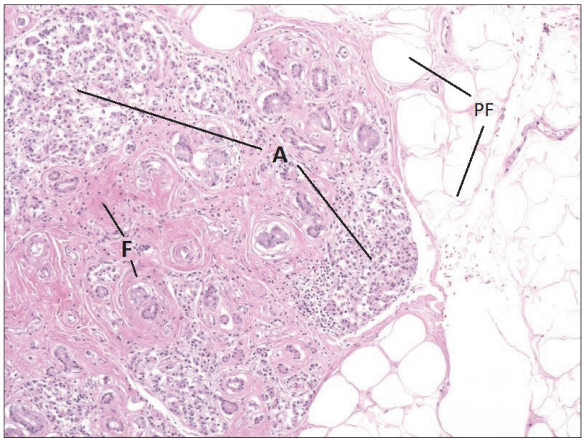

Figure 4 Pancreatic atrophy in a dog. Atrophic areas (A) and fibrotic areas (F) are interspersed. Peripancreatic fat (PF) can also be observed H&E stain; magnification: 40X *Courtesy of Dr. K. Burke*

(Newman et al., 2004; Richter, 2013). If pancreatic biopsies are collected, they should be taken from the margins of the organ as collection from within the gland may lead to injury of the duct system and/or major vessels (Spillmann et al., 2000). In spite of this, the danger of inducing pancreatitis during pancreatic biopsy has been reported to be very low (Spillmann et al., 2000; Harmoinen et al., 2002; Barnes et al., 2006; Kook et al., 2012). Collected samples may be graded using a number of grading schemes (Newman et al., 2006; Watson et al., 2007; Mansfield et al., 2012), which are based on the presence or absence of neutrophilic and/or lymphocytic inflammation, atrophy, edema, peripancreatic fat necrosis, and fibrosis

(**Figures 3,4**). Expected histologic changes in CP include the dilation of pancreatic ducts, parenchymal fibrosis, reduced size and number of acini, and lymphoplasmacytic infiltration, while on gross inspection the organ itself would appear shrunken, distorted, and nodular in appearance (McGavin et al., 2007).

2.2.5 Treatment

The management of chronic pancreatitis consists of removing any identified underlying cause as well as symptomatic care. Treatment is usually aimed at improving a patient's quality of life by use of analgesia, nutritional management, and addressing functional loss because often times the etiology is unknown (Watson, 2012).

Dogs presenting with vomiting or nausea may benefit from being treated with anti-emetics. Maropitant (Cerenia®) is usually well tolerated in dogs, effective in treating vomiting when used at 1mg/kg/d SC, (Xenoulis, 2013) and may also have some analgesic effects (Twedt, 2013).

Nutritional support with a low-fat diet is beneficial and should be attempted as soon as the patient has stopped vomiting (Steiner, 2008; Xenoulis, 2013). Depending on how far CP has progressed, and how much of the pancreatic parenchyma has been damaged, patients may show signs of EPI and may require enzyme replacement therapy (Watson, 2012).

Pain is thought to accompany all cases of canine pancreatitis but can be masked in some patients (Steiner, 2008). Therefore, analgesics should be considered, at least initially. Oral tramadol or butorphanol are reasonable choices for mild pain, but fentanyl patches are the only treatment option for outpatients with signs of severe abdominal pain (Xenoulis, 2013).

Patients with CP may present with an acute episode that is virtually indistinguishable from acute pancreatitis. These patients can be severely dehydrated and present with serious electrolyte and acid-base abnormalities. In such instance, other measures including fluid therapy and plasma may be required to stabilize the patient before long-term therapy can be initiated.

2.3 Synthetic serine protease inhibitor camostat mesilate FOY-305

Camostat mesilate (N,N-dimethylcarbamoylmethyl-4-(4-guanidinobenzoyloxy) phenylacetate methanesulfonate) is a synthetic low-molecular weight serine protease inhibitor. Experimental studies indicate it has pronounced inhibitory effects on trypsin, kallikrein, plasmin, thrombin, and C1-esterase enzyme cascades (Tamura et al., 1977; Sakaguchi, 1980; Bonner et al., 1987). In analogy to natural serine protease inhibitors (SERPINS), inhibition is caused by the induction of conformational changes via covalent linkage and distortion of the active site of target proteases (Huntington et al., 2000; Law et al., 2006).

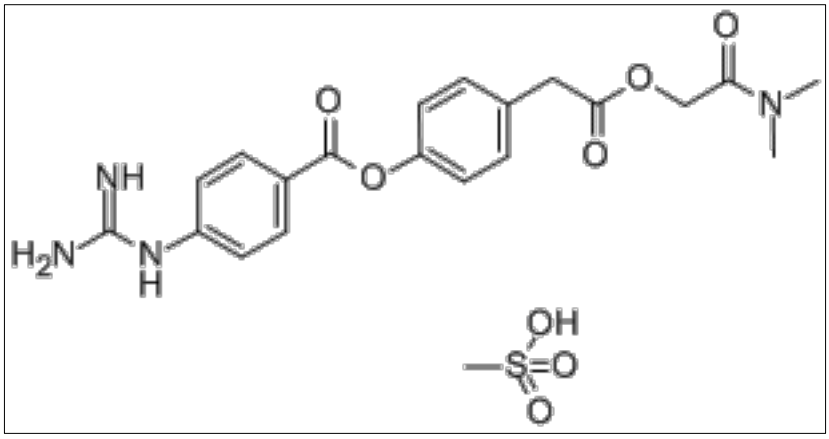

Figure 5 Chemical structure of N,N-dimethylcarbamoylmethyl-4-(4-guanidinobenzoyloxy) phenylacetate methanesulfonate, camostat mesilate, FOY-305 (Kretzschmar, 2014)

Camostat given to rats undergoes rapid hepatic transformation into 4-guanidinobenzoic acid (GBA), which lacks anti-protease activity (Ohki et al., 1980), and its active anti-proteolytic metabolite 4-(4-guanidino-benzoyloxy) phenylacetic acid (GBPA, FOY-251) (Beckh et al., 1987; Nishihata et al., 1988; Midgley et al., 1994). The main eliminatory organs of CM and its metabolites are the liver and kidneys, with renal excretion accounting for a minimum of 80% of the dose in dogs (Midgley et al., 1994). Hiraku showed that GBA and GBPA are also the main metabolites of CM in people (Hiraku, 1982).

Muryobayashi investigated the pharmacological effects of CM on general symptoms, the central and autonomic nervous system, the cardio-respiratory system, the urinary system, on antigenicity and local irritation in a variety of animal species. His experiments showed no

changes in general symptoms in mice and rats up to an oral dose of 500 mg/kg (Muryobayashi, 1980).

In Japan, CM has been in clinical use for humans for more than two decades (Tanaka et al., 1979; Hirono, 1980; Horiguchi, 1980; Ishii, 1984; Fukuda et al., 2009). In two double-blinded, randomized studies, patients with acute and chronic pancreatitis received CM or a placebo. CM was more effective at improving the primary end-points abdominal pain/tenderness and urine/serum amylase concentration when compared to a control group (Hirayama, 1980; Ishii, 1984). Significant gradual improvement of subjective symptoms including epigastric discomfort, tenderness, nausea and vomiting was also noted by Fujiwara, Hayawaka and Hirono in patients with acute and chronic pancreatitis that had received CM treatment (Fujiwara, 1980; Hayawaka, 1980; Hirono, 1980). Ishii applied varying stringency to enrollment criteria in two double-blinded multi-center studies that aimed at evaluating the efficacy of CM and found that beneficial effects are greater when patients with AP are excluded and enrollment is restricted to those with CP (Ishii, 1980; Ishii, 1984).

CM has also been used in people for the treatment of postoperative reflux esophagitis. A condition where reflux of bile acids and pancreatic enzymes after gastrectomy causes inflammation of the esophagus. Treatment with CM after experimental removal of the stomach and subsequent oesophagojejunostomy in rats results in reduced mucosal injury, ulceration and histopathological signs of inflammation of the esophagus when compared to untreated control groups (Kamiyasu et al., 1991; Kawabata, 1992; Imada et al., 1999). The underlying mechanism to CM's use in this particular situation is the disruption of trypsin-dependent enzyme cascades that result in esophageal mucosal damage. Data from 28 human patients indicate CM treatment after gastrectomy leads to positive changes in disease activity according to the Los Angeles classification and lowers trypsin activity in those study subjects where trypsin was found in esophageal lavage fluid (Kono et al., 2005).

Yet there are insufficient data regarding camostat's clinical efficacy in dogs. Additionally, the vast majority of pharmacology and toxicology studies was performed on laboratory rodents and employed various protocols to induce disease. A well-established rodent model for CP is the male Wistar Bonn/Kobori (WBN/Kob) rat that develops spontaneous pancreatitis at the age of 3 months (Mori et al., 2009). The histopathologic findings in these animals, i.e. fibrosis, destruction of parenchyma, infiltration of inflammatory cells, accurately mimic the findings generally associated with chronic

pancreatitis (Ohashi et al., 1990). Alternatively, pancreatic fibrosis can be induced in rats by repeated intraperitoneal application of diethyldithiocarbamate (DDC) (Matsumura et al., 2001).

In vitro and *in vivo* studies based on animal models and cell cultures have shown that camostat mesilate exerts anti-inflammatory and anti-fibrotic properties, which appear to be due to suppression of pro-inflammatory cytokines, the kinin-forming systems and pancreatic stellate cells (PSC) (Sugiyama et al., 1996; Su et al., 2001; Gibo et al., 2005; Jia et al., 2005). Kinins can be formed by free trypsin and are physiologically potent peptides that pharmacologically act as inflammatory mediators (Duchene, 2012). In a study by Obata, formation of kinins was suppressed *in vivo* in rats by CM (Obata, 1980).

PSCs have become a major target of chronic pancreatitis research because of their role in maintaining normal pancreatic architecture through fibrogenesis and matrix degradation. Using the WBN/Kob rat spontaneous pancreatitis model, Su et al investigated the effects of CM on CP and demonstrated that pancreas-associated protein (PAP), IL-6, and TGF-β gene expression were suppressed in treated animals when compared to an untreated control group (Su et al., 2001). A study by Gibo et al. investigated *in vivo* effects of oral CM on experimentally-induced CP in rats, as well as *in vitro* effects on isolated monocytes and PSCs. The production of monocyte chemo-attractant protein 1 (MCP-1) and TNF-α from isolated monocytes, as well as proliferation and MCP-1 production from PSCs decreased significantly, while histological examination revealed reduced pancreatic inflammation and fibrosis (Gibo et al., 2005). Histologically confirmed attenuation of CP in experimental rat models for chronic pancreatitis has also been reported by Jia (Jia et al., 2005; Jia et al., 2005), Emori, (Emori et al., 2005) and Sugiyama (Sugiyama et al., 1996). Notably, anti-fibrotic effects of CM have been demonstrated not only in the pancreas but in other organs as well. Two studies reported significant reductions in fibroblast activation in the liver and kidney, as well as decreased TGF-β1 levels when rats with induced CP were treated with a low dose of CM (Okuno et al., 2001; Morinaga et al., 2013). However, while there is mounting evidence that suppression of TGF-β leads to decreased fibroblastic action in various tissues, and thus decreases extra cellular matrix deposition, the mechanism by which this is achieved in the pancreas is not yet fully understood.

In rodents with induced CP, oral CM treatment led to significant increases in body weight and pancreas wet weight (Ichikawa, 1980; Shimoda I, 1993; Su et al., 2001; Gibo et al., 2005). An increase in pancreas wet weight was observed in rats after oral treatment with 100, 235, 550 and 1300 mg/kg of CM (Matsuoka, 1980). Conversely, a study based on a type-2

diabetes model in cholecystokinin-1 deficient Otsuka Long-Evans Tokushima Fatty (OLETF) rats reported a loss of body weight associated with long-term (44 to 60 weeks) CM treatment (Jia et al., 2005). However, in this study rats were fed a diet supplemented with 200mg of camostat per 100g. Thus the exact CM dose adjusted for body weight is unknown. The cause for the increase in body weight is unclear. Histologic evaluation of the pancreas after CM treatment showed hypertrophy and increased numbers of zymogen granules (Matsuoka, 1980). This may explain findings of one study, where a significant increase in exocrine secretions was observed in CM-treated WBN/Kob rats with CP (Sugiyama et al., 1996).

Although the specific mechanism by which CM has this effect is unknown, mounting evidence also suggests that CM suppresses pancreatitis-related visceral pain. The basis for these observations may possibly be explained with the discovery of trypsin's role in mediating inflammatory pain in acute pancreatitis. The observed behavioral pain response and activation of spinal nociceptive receptors produced in rats if trypsin is injected into the pancreatic duct (Hoogerwerf et al., 2004) are possibly mediated via the proteinase-activated receptor 2 (PAR-2) (Hoogerwerf et al., 2001; Hoogerwerf et al., 2004; Ceppa et al., 2011).

Ishikura et al. induced acute pancreatitis in mice and rats via intra-peritoneal caerulin infusion and intra-ductular injection of trypsin. While oral administration of CM decreased c-Fos markers of trypsin-induced nociception in rats, pancreatitis-associated hyperalgesia was significantly reduced in mice (Ishikura et al., 2007). Because anti-allodynic effects observed in mice outweighed anti-inflammatory ones, the authors suggested that "…the former are not necessarily secondary to the latter".

There are currently no data on therapeutic dosage ranges for dogs. The only data on CM and its effects in dogs stem from a sub-acute toxicity study which investigated 4 different dosages of CM in a cohort of Beagle dogs. Using urinalysis, serum biochemistry profiles, necropsy, and histopathology, this study found no significant changes in dogs given an oral dose of 10 mg/kg/d (10 mg/kg q 24 hrs). An increase in white blood cell count was observed in dogs that received 30 mg/kg/d (30 mg/kg q 24 hrs) and 100 mg/kg/d (50 mg/kg q 12 hrs). This was not associated with histologic changes and the factors leading to this observation are unclear. Oral administration of 300 mg/kg/d (100 mg/kg q 8 hrs) on an empty stomach resulted in a significant decrease in food intake, severe vomiting and hemorrhage of the gastrointestinal tract. In this group dogs became debilitated and 2 out of 3 subjects died (Matsuoka, 1980).

2.4 C-reactive protein and S100A12 - serum markers of inflammation

2.4.1 C-reactive protein

C-reactive protein (CRP) is a highly conserved, nonspecific, acute phase plasma protein. It is synthesized in hepatocytes in response to inflammation, infection, or tissue damage (Black et al., 2004), with serum levels increasing up to 1000-fold (Eckersall et al., 2010). Therefore, often times CRP will increase even before changes in CBC become obvious (Eckersall et al., 1988). In the event of tissue injury or necrosis, CRP binds to certain surface proteins and activates the complement system, which in turn triggers phagocytotic clearance by macrophages. In the clinical setting, CRP has been used for grading and monitoring of dogs with inflammatory bowel disease. The decision to utilize this assay was made under consideration of CM's purported anti-inflammatory properties and because no data are available concerning CM's impact on serum C-reactive protein concentrations in dogs with chronic pancreatitis.

2.4.2 Serum S100A12

S100A12 (aka, calgranulin C) is one of three members of the calgranulin family of highly conserved S100 proteins and plays an important role in the innate immune system (Hsu et al., 2009). S100A12 was first identified and is predominantly found in the cytosolic compartment of neutrophils and monocytes (Guignard et al., 1995; Vogl et al., 1999). S100A12 and the other calgranulins S100A8 and S100A9 share an EF-hand motif and the capacity to bind divalent calcium, copper, and zink ions (Heizmann, 2002). Binding these ions acts as a molecular switch that induces conformational changes obligatory for the interaction with target domains (Xie et al., 2007). S100A12 secreted by neutrophils or monocytes in response to cellular stress (i.e., inflammation) binds Ca^{2+}-ions and thus creates hydrophobic surface sites, which then act as ligands for the transmembrane receptor for advanced glycation products (RAGE) (Xie et al., 2007). Ligation mediates a pro-inflammatory, NF-κβ-dependent, stimulus (Xie et al., 2007) that precipitates the induction of oxidative stress and activates intracellular signaling cascades, leading to the release of pro-inflammatory cytokines (Tak et al., 2001; Yang et al., 2007; Yilmaz et al., 2011).

While the diagnostic usage of S100A12 in dogs is, at this point, in its infancy, it has been recognized as a specific and sensitive marker for various inflammatory disorders in humans,

including IBD (Foell et al., 2003; Kaiser et al., 2007; Wittkowski et al., 2008) and rheumatoid arthritis (Foell et al., 2004). Interestingly, calgranulin C is thought to induce a RAGE–associated positive feedback loop that leads to the perpetuation of inflammatory responses and would therefore play a direct role in the pathogenesis of pancreatitis (Gebhardt et al., 2008).

Recently, an assay for the measurement of S100A12 in canine feces and serum has been developed and analytically validated (Heilmann et al., 2011). Preliminary results using this assay have been promising. One clinical study showed a positive correlation between the severity of colonic inflammation and fecal S100A12 concentrations in dogs with IBD (Heilmann et al., 2014). Serum S100A12 concentrations are increased in humans with mild to severe acute pancreatits (Farkas Jr et al., 2014). However, to the author's knowledge studies on the correlation of S100A12 with disease activity in canine pancreatitis are lacking. Therefore, one aim of the present study was to measure serum concentration of S100A12 in dogs with acute or chronic pancreatitis and investigate any effect of camostat mesilate on this biomarker of inflammation.

3 OBJECTIVES

The primary goal of this study was to determine the effect of two different dosages (12 mg/kg/d at a rate of 4 mg/kg q 8 hrs; and 24 mg/kg/d at a rate of 8 mg/kg q 8hrs) of camostat mesilate in 31 dogs with suspected naturally occurring CP in a non-randomized, pre-post interventional study in order to assess the impact of this compound on acinar cell damage (i.e., as estimated by measurement of serum cPLI concentration), serum concentrations of systemic markers of inflammation (i.e., S100A12, CRP) and a serum marker of fibrosis (i.e., TGF-β1). Secondary objectives included evaluation of treatment effects on overall health status, quality of life, and clinical signs employing serum biochemistry profiles, complete blood counts, and veterinarian post-treatment follow-up questionnaires.

4 MATERIALS AND METHODS

4.1 Study design

The study was configured as a modified two-group, non-controlled, non-blinded, non-randomized pretest-posttest interventional design, with two pretests (pre-treatment test 1/data base screening, followed by pre-treatment test 2, followed by intervention/treatment, followed by post-treatment test). The study population was distributed throughout the United States and consisted of privately owned dogs with suspected naturally occurring chronic pancreatitis.

Pre-treatment measurement 1 was not related to the study and either a patient-side SNAP cPL test, or an accession submitted to the Gastrointestinal Laboratory at Texas A&M for measurement of cPLI. Pre-treatment measurement 2 included serum concentrations of cPLI, cTLI, cobalamin, and folate, as well as a serum biochemistry profile and a CBC. Patients were split in two groups. One group received the trial drug at a total dose of 12 mg/kg/d (4 mg/kg q 8hrs), while another group received a total dose of 24 mg/kg/d (8 mg/kg q 8hrs). Enrollment of patients into the two groups was conducted sequentially, i.e., recruitment for the 24 mg/kg group was initiated only after treatment of all patients in the 12 mg/kg group had been completed. The length of the treatment period was set at 21 to 31 days and individual diets remained unchanged for the duration of the trial. Serum biochemistry profiles, CBC, and concentrations of cPLI, cTLI, cobalamin, and folate were measured

immediately post-treatment. The trial was concluded with a follow-up questionnaire for participating/submitting veterinarians. These were supplemented with daily logs kept by the patient owners. In order to reduce inter- and intra assay bias, aliquots of all pre-treatment 2 and post-treatment samples were stored at -20 °C and serum TGF-β1, serum S100A12, cPLI and CRP reevaluated after completion of the trial as a batch measurement.

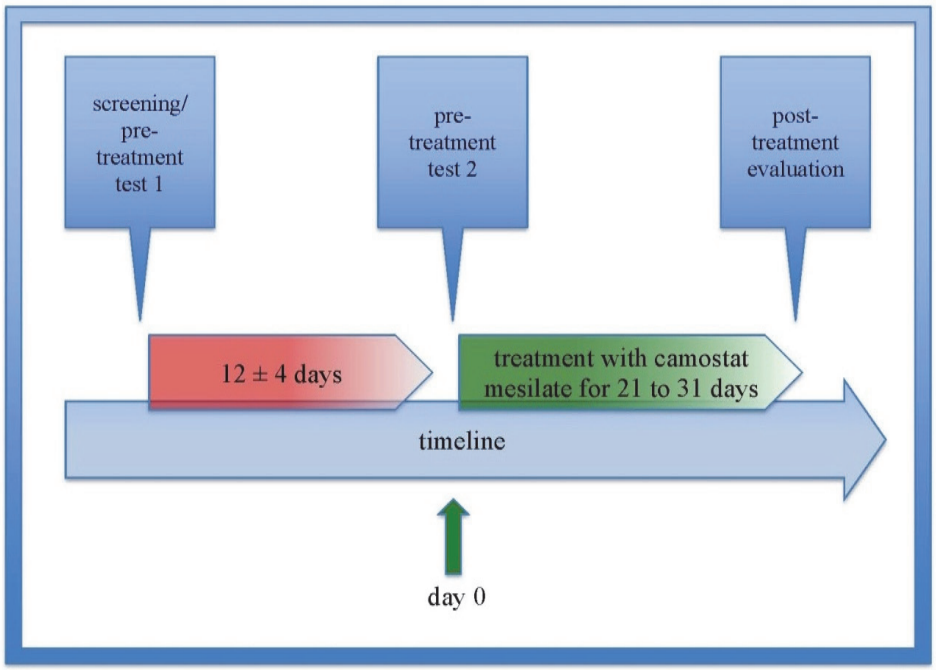

Figure 6 Study design and timeline

4.2 Study subjects

Pre-treatment test 1: Eligible dogs were identified between June 2012 and May 2014 by daily screening of the Gastrointestinal Laboratory database for cPLI results indicative of pancreatitis (serum cPLI concentration ≥400µg/L). Veterinarians directly referred additional patients to one of the investigators based on positive patient-side SNAP cPL® (IDEXX Laboratories, Westbrook, ME, USA) results and concomitant clinical signs.

Pre-treatment test 2: 12 ± 4 days after ascertaining individual suitability with the referring veterinarian, a serum biochemistry profile and CBC were performed, and serum cPLI concentrations were re-evaluated (n = 160 ± 5 dogs). Serum cPLI concentrations above ≥ 400 µg/L at the time of the pre-treatment tests 1 and 2 were considered indicative of chronic

pancreatitis. Patients were eligible for enrollment if one of the following inclusion criteria were satisfied:

- Pre-treatment test 1 plus pre-treatment test 2 cPLI concentrations ≥ 400 µg/L
- Positive patient-site SNAP test plus pre-treatment test 2 cPLI concentration ≥ 400 µg/L

Patients with clinical and/or laboratory signs indicative of systemic disease suspected to potentially influence the study outcome (i.e., hepatic, renal, or major other systemic disease) and patients with serum triglyceride concentrations ≥500 mg/dL were excluded.
All phlebotomies were carried out at participating veterinary practices. Pet owners administered oral camostat mesilate. Owner consent was required for enrollment into the trial. The animal care and use protocol was reviewed and approved by the Texas A&M Institutional Animal Care and Use Committee (Animal Use Protocol #2012-042).

4.3 Interventions/treatment

One group was treated with 12 mg/kg/d (4 mg/kg q 8hrs) for 21 to 31 days, while a second group was treated with 24 mg/kg/d (8 mg/kg q 8hrs) for the same time period (i.e., 21 to 31 days). Camostat mesilate was formulated into capsules by STASON Pharmaceuticals, Irvine, CA, USA, using hard gelatin capsules in sizes #2, #3, and #4 (depending on patient BW) from Capsugel, Morristown, NJ, USA.

4.4 Sample processing

All samples were taken after a minimum of withholding food for 12 hours. Blood samples for the second time point pre-treatment were collected 12 ± 4 days after the first pre-treatment sample. Post-treatment samples were collected at the very end of the treatment period, while patients were still receiving CM treatment. All samples were refrigerated, and shipped overnight on ice packs. Sample aliquots were stored at -20°C until final cPLI, CRP, S100A12, and TGF-β1 sample batch analysis. All measurements were conducted by personnel of the Gastrointestinal Laboratory at Texas A&M University (i.e. serum

biochemistry, folate, cobalamin, cTLI, cPLI, CRP) or at the Texas Veterinary Medical Diagnostic Laboratory (i.e., CBC), if not indicated otherwise.

4.5 Outcome measures

Outcome measures included serum concentrations of cPLI, cTLI, cobalamin, folate, CRP, S100A12, and TGF-β, as well as complete blood counts, serum biochemistry profiles, and quality of life questionnaires. Complete blood counts were performed by the Texas Veterinary Diagnostic Laboratory, College Station, TX, USA. All tests, excluding CRP, S100A12, and TGF-β1, were performed 4±1 days before treatment was initiated and at the very end of the treatment period.

4.5.1 Serum cPLI concentrations

Serum concentrations of cPLI were measured at the Gastrointestinal Laboratory at Texas A&M University using the Spec cPL® assay (IDEXX Laboratories, Westbrook, ME, USA). The Spec cPL assay is a solid phase ELISA that has been analytically validated for the measurement of canine pancreas-specific lipase in serum with an established reference interval of 0 -200 µg/L (Steiner et al., 2003; Huth et al., 2010). Samples with cPLI results exceeding the working range of the Spec cPL assay (> 1000 µg/L) were diluted and re-analyzed. An initial cPLI of > 400 µg/L at the pre-treatment measurement 1 was considered indicative of current pancreatitis. Patients with repeatedly increased cPLI > 400 µg/L at pre-treatment measurement 1 and 2 were considered to have chronic pancreatitis. Treatment efficacy was assessed by comparing pre-and post-treatment cPLI concentrations. A reduction in cPLI concentration was considered an improvement. Conclusive cPLI batch analysis was conducted by the investigator.

4.5.2 Serum cTLI concentrations

Serum cTLI concentrations (RI: 5.7 - 45.2 µg/L) were measured at the Gastrointestinal Laboratory at Texas A&M University (Gastrointestinal Laboratory at Texas A&M, 2014) (Canine TLI double antibody, Siemens Healthcare Diagnostics, Los Angeles, CA, USA) in order to assess impact of treatment on exocrine pancreatic function.

4.5.3 C-reactive protein

Serum concentrations of canine CRP (RI: 0 – 7.6 mg/L) were measured at the Gastrointestinal Laboratory at Texas A&M University using a solid phase sandwich immunoassay previously validated in dogs (Tridelta Development Limited, Maynooth, CO. Kildare, Ireland) (Gastrointestinal Laboratory at Texas A&M, 2014).

4.5.4 Serum S100A12 concentrations

Canine serum S100A12 concentrations (RI: 56 – 331 µg/L) were measured at the Gastrointestinal Laboratory at Texas A&M University using an in-house solid phase ELISA that has been analytically validated for canine serum samples (Heilmann, 2014).

4.5.5 Serum TGF-β 1 concentrations

Canine serum TGF-β1 concentrations (RI: 38 – 72 ng/mL) were measured at the Gastrointestinal Laboratory at Texas A&M University using a mouse/rat/porcine/canine TGF-β1 solid phase ELISA that had been validated for the measurement of serum concentrations of TGF-β1 (R&D Systems, 2014) (Quantikine® ELISA, R&D Systems, Minneapolis, NE, USA). All TGF- β 1 measurements were conducted by the investigator.

4.5.6 Serum cobalamin and folate concentrations

Serum cobalamin (RI: 251 - 908 ng/L) (Gastrointestinal Laboratory at Texas A&M, 2014), and serum folate (RI: 7.7 - 24.4 µg/L) (Gastrointestinal Laboratory at Texas A&M, 2014) concentrations were measured at the Gastrointestinal Laboratory at Texas A&M University (Immulite 2000, Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA).

4.5.7 Complete blood count

Complete blood counts (red blood cell count, HCT, MCV, MCH, MCHC, hemoglobin concentration, absolute and differential white blood cell count, and platelet count), as well as

blood smear analysis (platelet estimate, red blood cell morphology, white blood cell morphology, hemoparasites) were evaluated at the Texas Veterinary Medical Diagnostic Laboratory (TVMDL). A Cell-Dyn 3700 hematology analyzer (Abbott Laboratories, Abbott Park, Il 60064 USA) was used for complete blood counts. Fresh blood smears were stained using Wright-Giemsa stain and microscopically evaluated with 100x magnification oil immersion.

4.5.8 Serum biochemistry

Serum chemistry profiles (glucose, blood urea nitrogen, creatinine, calcium, phosphorus, albumin, total protein, total bilirubin, alkaline phosphatase, alanine amino transferase, aspartate amino transferase, γ-glutamyl transferase, cholesterol, triglycerides, globulin, sodium, potassium, and chloride) were performed at the Gastrointestinal Laboratory at Texas A&M University on a SIRRUS analyzer (Stanbio, Boerne, TX, USA).

4.5.9 Follow-up questionnaires

After completion of the study, veterinarians were asked to evaluate changes in their patient's overall subjective quality of life. The questionnaire was set-up in closed-ended question format with 3 possible answers (no change, improvement, decline) without grading degrees and the option to decline grading was offered (**Fig. 31**). The follow-up questionnaire also asked for post-treatment body weight, as well as details (date, route of application, dose) of possible pre-treatment cobalamin supplementation. Additional treatments and individual diets were not assessed.

In order to aid grading, facilitate communication, and collaboration between veterinarians and pet owners, pet owners were instructed to maintain a daily log for the duration of CM treatment (**Fig. 32**). In this log varying grades of abdominal pain, attitude, drinking, and parameters pertaining to elimination corresponded to a numerical qualifier.

4.5.10 Statistical analysis

Results from pre-treatment 2 measurements and post-treatment measurements were statistically analyzed using Prism 6 (GraphPad Software, La Jolla, CA, USA). The D'Agostino-Pearson omnibus test was used to evaluate all datasets for Gaussian distribution. Groups were compared using a matched pairs t-test for normally distributed data sets and a Wilcoxon matched pairs test when Gaussian distribution could not be assumed. Mean values and standard deviation are reported for data sets with Gaussian distribution. Median and minimum/maximum values are reported for data sets where Gaussian distribution could not be assumed. The significance threshold was set at $p=0.05$. Spearman nonparametric correlation was used to calculate correlations between duration of treatment and differences between pre-treatment and post-treatment cPLI concentrations when Gaussian distribution could not be assumed. Spearman's r was computed for X versus every Y dataset. Pearson correlation was used for normally distributed data sets. The significance threshold was set at $p=0.05$.

5 RESULTS

5.1 Signalment

The 12 mg/kg/d group consisted of 1 intact female, 7 spayed females, and 4 neutered males with an average age [range] of 10.5 [2 – 14] years. The 24 mg/kg/d group consisted of 5 intact females, 8 spayed females, and 6 neutered males with an average age [range] of 10.1 [3 – 15] years. Overall, 21/31 dogs (68%) were 10 years or older and 15/31 dogs (48%) were spayed females. Dachshunds (n=6) and mixed breed dogs (n=6), followed by Miniature Schnauzers (n=4), and Cavalier King Charles Spaniels (n=2) were the most common breeds.

Table 3: Signalment 12 mg/kg/d, n=12

Breed	Age			Sex			
	Young	Middle-aged	Old	F	FS	M	MN
Dachshunds n=3	0	1	2	0	1	0	2
Mixed breed n=2	0	0	2	0	1	0	1
Cavalier King Charles n=2	0	1	1	0	1	0	1
Other n=5	1	1	3	1	4	0	0
Overall n=12	1	3	8	1	7	0	4

Young = 0-4 yrs; Middle-aged = 5-8 yrs; Old = > 9 yrs; F = intact female; FS = spayed female; M = male; MN = neutered male

Table 4: Signalment 24 mg/kg/d, n=19

Breed	Age			Sex			
	Young	Middle-aged	Old	F	FS	M	MN
Dachshunds n=3	1	0	2	1	1	0	1
Miniature Schnauzers n=3	0	2	1	2	1	0	0
Mixed breed n=4	1	0	3	1	1	0	2
Other n=9	0	2	7	1	5	0	3
Overall n=19	2	4	13	5	8	0	6

Young = 0-4 yrs; Middle-aged = 5-8 yrs; Old = > 9 yrs; F = intact female; FS = spayed female; M = male; MN = neutered male

5.2 Adverse drug reactions

Overall, side effects of treatment were noticed in 6 of 31patients enrolled. Two dogs treated with 12 mg/kg/d (2/12; 17%) presented with increased flatulence, but completed the treatment course. In the group of dogs treated with 24 mg/kg/d, one dog (1/19; 5%) exhibited signs of pruritus in one front paw and one dog (1/19; 5%) presented with malodorous and increased flatulence. Both dogs completed treatment. One patient was withdrawn by the owner from the trial due to diarrhea and vomiting (1/19; 5%). Marked post-treatment increases in liver enzyme activities (AST: 36 U/L to 178 U/L; RI: 12 – 48; GGT: 5 U/L to 177 U/L; RI: 0 – 12 U/L) were observed in one dog (1/19; 5%). No other side effects or serum biochemistry and whole blood count abnormalities suggestive of an adverse drug reaction were observed in either group.

5.3 Drop-outs

Out of 14 dogs initially enrolled in the 12 mg/kg treatment group, 2 (14%) dogs were euthanized due to pre-existing conditions unrelated to the study und unknown to the investigator and submitting/participating veterinarians at the time of enrollment and were thus unavailable for post-treatment follow-up. One (1/14) dog (age: 12 years, sex: spayed female, breed: Shetland Sheepdog) was euthanized due to a transitional cell carcinoma of the urinary bladder. One (1/14) dog (age: 12 years, sex: spayed female, breed: Cocker Spaniel) was euthanized due to symptoms associated with the nervous system that were most likely caused by a large intracranial mass, as reported by the participating veterinarian. Out of 22 patients initially enrolled in the 24 mg/kg group 3 (14%) dogs were unavailable for post-treatment follow-up. One (1/22) patient owner decided to abandon the trial after receiving the trial medication but before treatment had been initiated. One (1/22) owner failed to appear for post-treatment testing and one (1/22) owner discontinued the trial when the dog experienced increased diarrhea and vomiting. In total, 5 of 36 (14%) patients that were initially enrolled dropped out of the study and were unavailable for post-treatment follow up.

5.4 Serum cPLI concentrations

Serum cPLI concentrations did not change significantly between pre-treatment (median: 501 µg/L; range: 344 to 6,052 µg/L) and post-treatment (median: 453 µg/L; range: 125 to 2,836 µg/L) concentrations in the 12 mg/kg/d group (n=12; p=0.9697). In this group an individual cPLI decrease was observed in 6/12 (50%) and an increase in 6/12 (50%) patients. However, a statistically significant decrease in serum cPLI concentration was observed between pre-treatment (median: 847 µg/L; range: 414 to 2,024 µg/L) and post-treatment (median: 520; range: 193 to 2,580 µg/L) in the 24 mg/kg/d group (n=19; p=0.016). In this group 14/19 (74%) showed a decrease in serum cPLI concentration, while 5/19 (26%) of patients showed an increase. A statistically significant decrease between pre-treatment (median: 690 µg/L; range: 344 to 6,052 µg/L) and post-treatment (median: 514 µg/L; range: 125 to 2,836 µg/L) serum cPLI concentrations was also observed for all treated dogs combined (n=31; p=0.0409). In this group decrease of serum cPLI concentration was observed in 20/31 (64%) dogs, while an increase was observed in 11/31 (36%) patients. There were no significant correlations between treatment duration and differences between pre-treatment and post-treatment serum cPLI concentrations for the 12 mg/kg/d group (n=12; ρ=0.04; p=0.91), the 24 mg/kg/d group (n=19; ρ=0.195; p=0.42), or all treated dogs combined (n=31; ρ=-0.0013; 0.94).

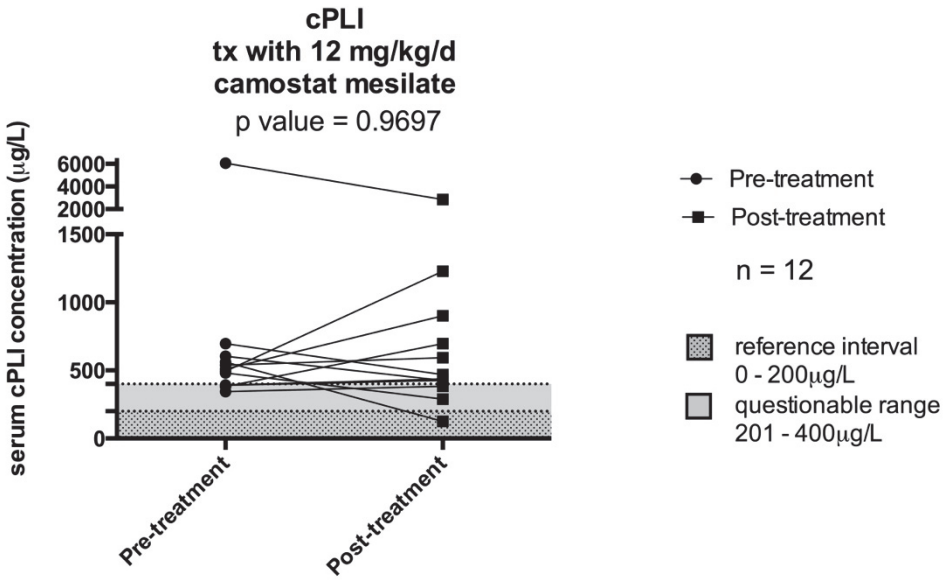

Figure 7 cPLI concentration tx 12 mg/kg/d This Figure shows serum cPLI concentrations (as measured by Spec cPL®) in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare values before and after treatment. There was no statistically significant difference between pre-treatment (median: 501 µg/L; range: 344 – 6,052 µg/L) and post-treatment (median: 453 µg/L; range: 125 – 2,836 µg/L; n = 12; p-value: 0.9697) cPLI concentrations.

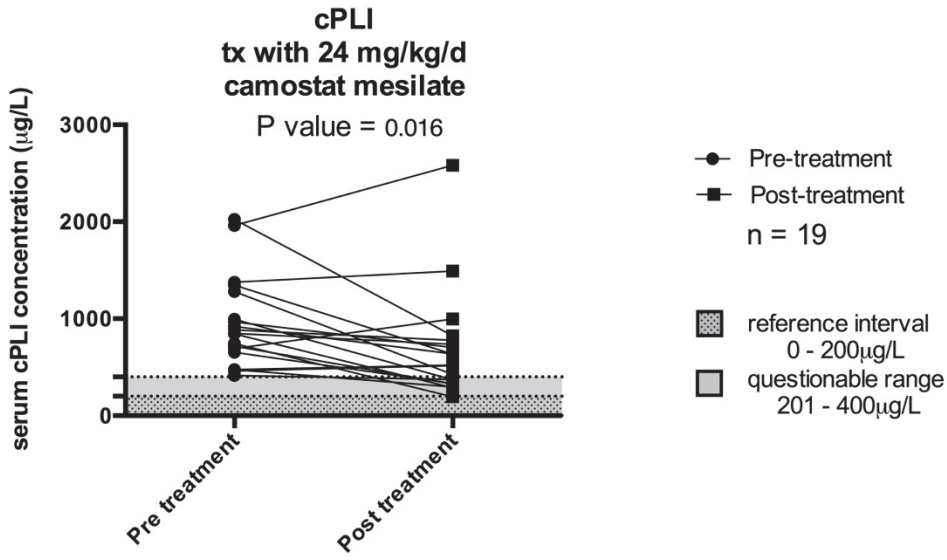

Figure 8 cPLI concentration tx 24 mg/kg/d This Figure shows serum cPLI concentrations (as measured by Spec cPL®) in dogs with suspected chronic pancreatitis before and after treatment with 24 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare values before and after treatment. There was a statistically significant decrease between pre-treatment (median: 847 µg/L; range: 414 to 2,024 µg/L) and post-treatment (median: 520; range: 193 to 2,580 µg/L; n=19; p-value: 0.016) cPLI concentrations.

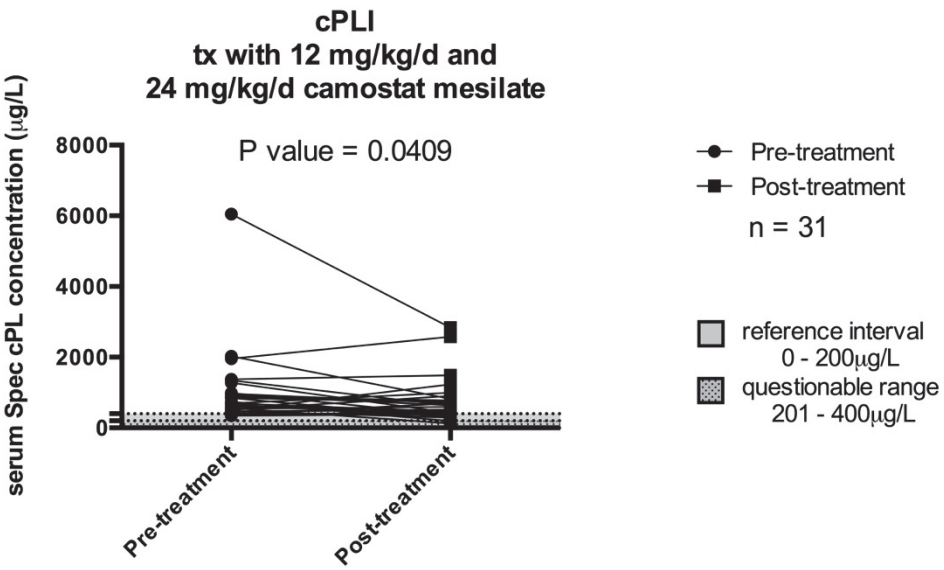

Figure 9 Serum cPLI concentrations tx 12 mg/kg/d and 24 mg/kg/d combined This Figure shows serum cPLI concentrations (as measured by Spec cPL®) in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg and 24 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare values before and after treatment. There was a statistically significant decrease between pre-treatment (median: 690 µg/L; range: 344 to 6,052 µg/L) and post-treatment (median: 514 µg/L; range: 125 to 2,836 µg/L; n=31; p-value: 0.0409) serum cPLI concentrations.

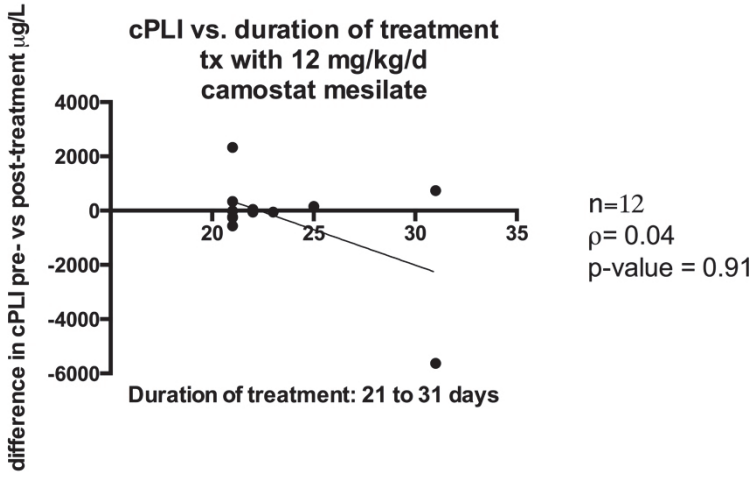

Figure 10 Duration of treatment and differences in serum cPLI concentrations before and after treatment for the 12 mg/kg treatment group. This Figure shows the correlation between the duration of treatment and the difference in serum cPLI concentration (as measured by Spec cPL®) in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg of camostat mesilate. Spearman nonparametric correlation was used to measure correlations of treatment duration and cPLI concentration. There was no statistically significant correlation between treatment duration and decrease in serum cPLI concentration (n=12; r_s= 0.04; p-value: 0.91).

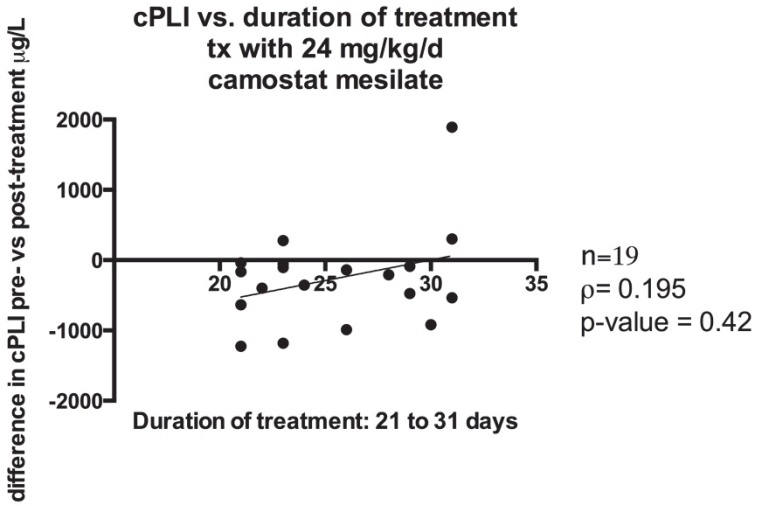

Figure 11 Duration of treatment and differences in serum cPLI concentrations before and after treatment for the 24 mg/kg treatment group. This Figure shows the correlation between the duration of treatment and the difference in serum cPLI concentration (as measured by Spec cPL®) in dogs with suspected chronic pancreatitis before and after treatment with 24 mg/kg of camostat mesilate. Spearman nonparametric correlation was used to measure correlations of treatment duration and cPLI concentration. There was no statistically significant correlation between treatment duration and decrease in serum cPLI concentration (n=19; r_s= 0.195; p-value: 0.42)

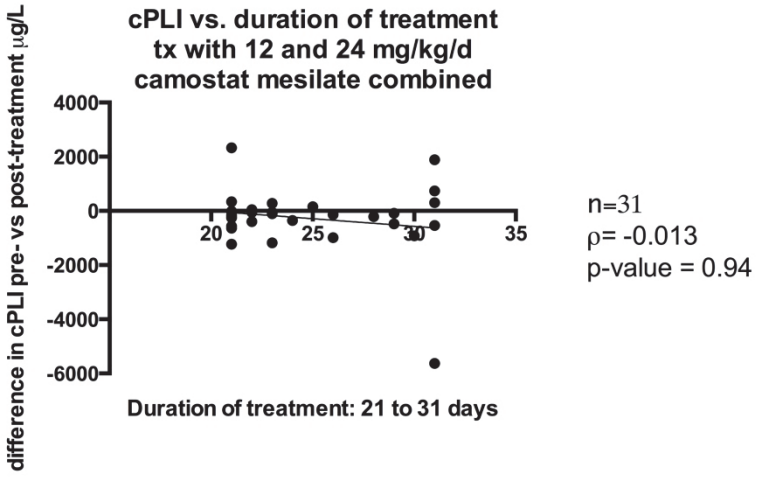

Figure 12 Duration of treatment and differences in serum cPLI concentrations before and after treatment for 12 mg/kg and 24 mg/kg treatment group This Figure shows the correlation between the duration of treatment and difference in serum cPLI concentration (as measured by Spec cPL®) in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg and 24 mg/kg of camostat mesilate. Spearman nonparametric correlation was used to measure correlations of treatment duration and cPLI concentration. There was no statistically significant correlation between treatment duration and decrease in serum cPLI concentration (n=31; r_s= 0.013; p-value:0.94)

5.5 Serum cTLI concentrations

Serum cTLI concentrations did not change significantly between pre-treatment (median: 21.7 µg/L; range: 19.5 to 92 µg/L) and post-treatment (median: 27.6 µg/L; range: 17.2 to 54.8 µg/L) concentrations in the 12 mg/kg/d group (n=11; p=0.831). Also, no significant changes in serum cTLI concentrations were observed between pre-treatment (median: 34.7 µg/L; range: 14.9 to 101µg/L) and post-treatment (median: 25.5 µg/L; range: 5.7 to 101 µg/L) values in the 24 mg/kg/d group (n=19; p=0.353). Finally, no significant changes in serum cTLI concentrations were observed between pre-treatment (median: 32.2 µg/L; range: 14.9 to 101 µg/L) and post-treatment (median: 26.1 µg/L; range: 5.7 to 101 µg/L) values for all treated dogs combined (n=30; p=0.36).

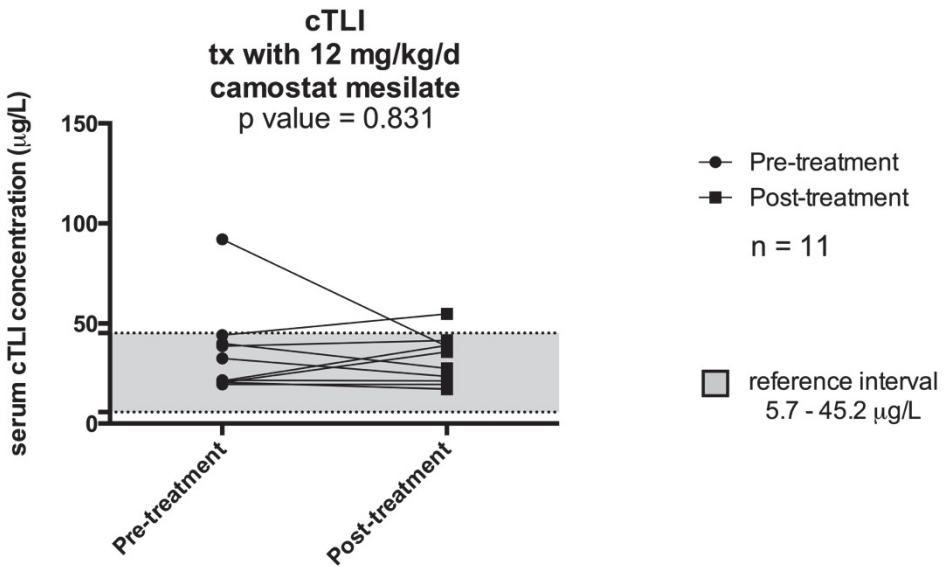

Figure 13 Serum cTLI concentrations tx 12 mg/kg/d This figure shows serum cTLI concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare the values before and after treatment. There was no significant difference between pre-treatment (median: 22 µg/L; range: 20 to 92 µg/L) and post-treatment (median: 28 µg/L; range: 17 to 55 µg/L; n = 11; p-value = 0.8311) cTLI concentrations.

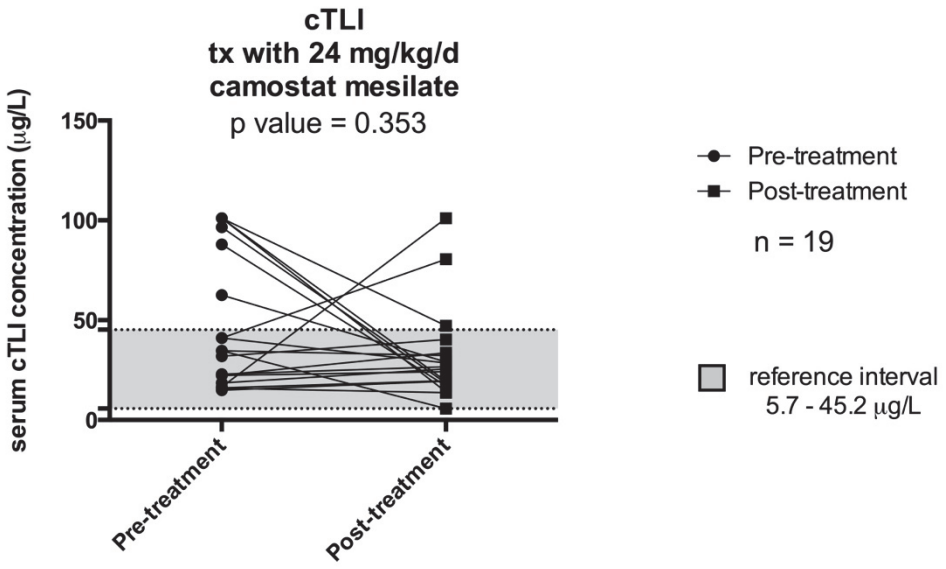

Figure 14 Serum cTLI concentration tx 24 mg/kg/d This figure shows serum cTLI concentrations in dogs with suspected chronic pancreatitis before and after treatment with 24 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare the values before and after treatment. There was no significant difference between pre-treatment (median: 34.7 µg/L; range: 14.9 to 101 µg/L) and post-treatment (median: 25.5 µg/L; range: 5.7 to 101 µg/L; n = 19; p-value = 0.353) cTLI concentrations.

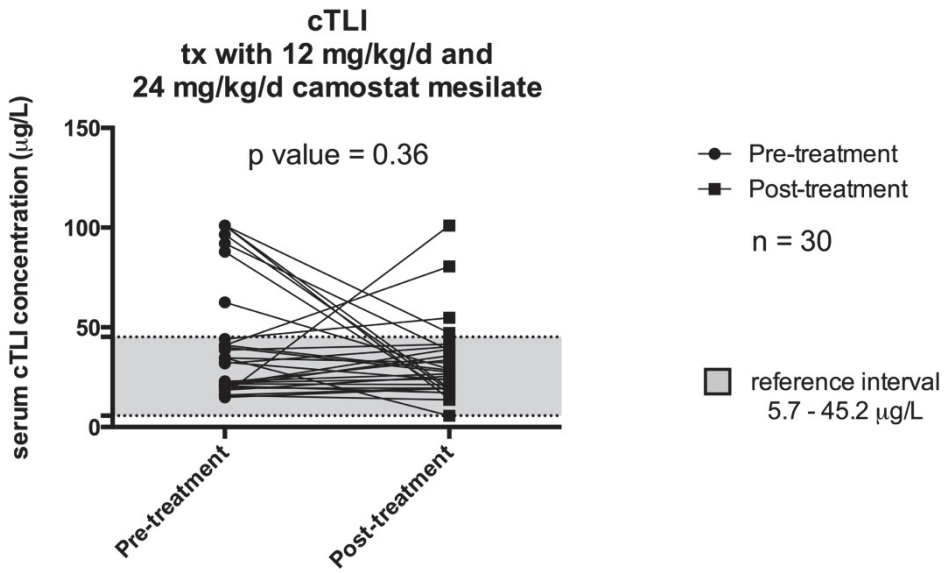

Figure 15 Serum cTLI concentrations tx 12 mg/kg/d and 24 mg/kg/d combined This figure shows serum cTLI concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg and 24 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare the values before and after treatment. There was no significant difference between pre-treatment (median: 32.2 µg/L; range: 14.9 to 101 µg/L) and post-treatment (median: 26.1 µg/L; range: 5.7 to 101 µg/L; n = 30; p-value = 0.36) cTLI concentrations.

5.6 Serum C-reactive protein concentrations

Serum CRP concentrations did not change significantly between pre-treatment (median: 0.8 mg/L; range: 0.1 to 30.3 mg/L) and post-treatment (median: 0.6 mg/L; range: 0.0 to 9.4 mg/L) concentrations in the 12 mg/kg/d group (n=12; p=0.1543). Serum CRP concentrations also did not change significantly between pre-treatment (median: 0.1 mg/L; range: 0.1 to 16.0 mg/L) and post-treatment (median: 0.1 mg/L; range: 0.1 to 6.8 mg/L) concentrations in the 24 mg/kg/d group (n=19; p=0.1475). However, a significant decrease between pre-treatment (median: 0.4 mg/L; range: 0.1 to 30.3 mg/L) and post-treatment (median: 0.1 mg/L; range: 0.1 to 9.4 mg/L) serum CRP concentrations was observed for all treated dogs combined (n=31; p=0.0281).

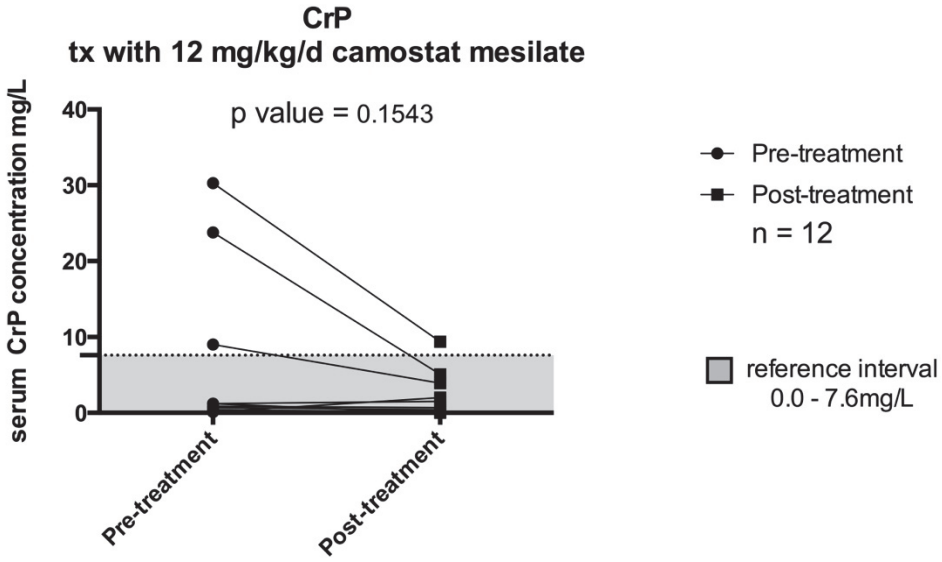

Figure 16 Serum CRP concentration tx 12 mg/kg/d This figure shows serum CRP concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare the values before and after treatment. There was no significant difference between pre-treatment (median: 0.8 mg/L; range: 0.1 to 30.3 mg/L) and post-treatment (median: 0.6 mg/L; range: 0.0 to 9.4 mg/L; n=12; p-value=0.1543) CRP concentrations.

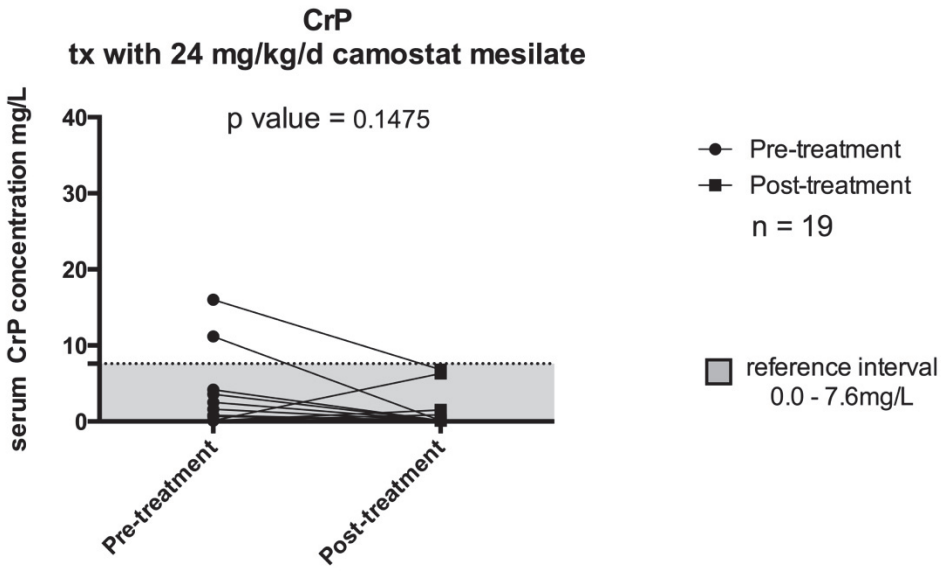

Figure 17 Serum CRP concentration tx 24 mg/kg/d This figure shows serum CRP concentrations in dogs with suspected chronic pancreatitis before and after treatment with 24 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare the values before and after treatment. There was no significant difference between pre-treatment (median: 0.1 mg/L; range: 0.1 to 16.0 mg/L) and post-treatment (median: 0.1 mg/L; range: 0.1 to 6.8 mg/L; n=19; p-value=0.1475) CRP concentrations.

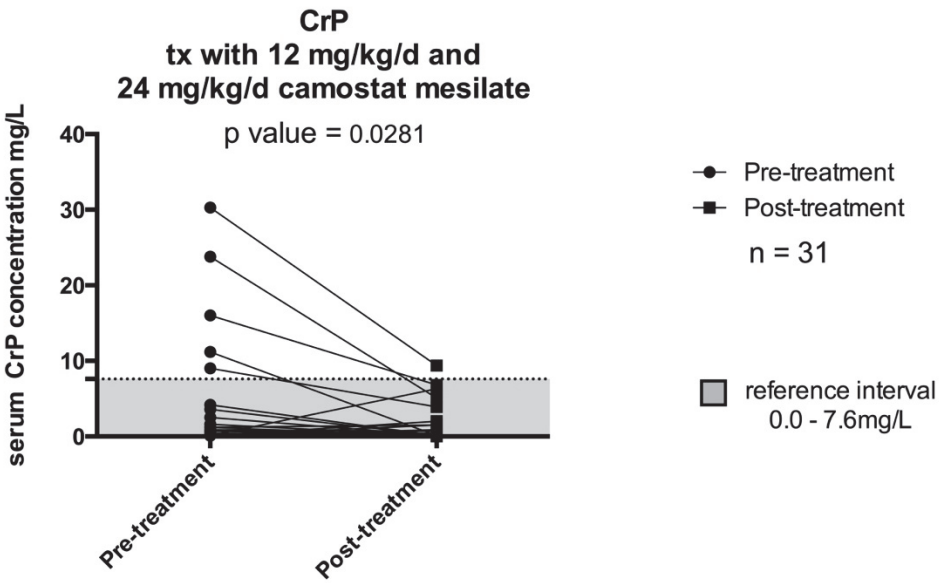

Figure 18 Serum CRP concentrations tx 12 mg/kg/d and 24 mg/kg/d combined This figure shows serum CRP concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg and 24 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare the values before and after treatment. There was a significant decrease between pre-treatment (median: 0.4 mg/L; range: 0.1 to 30.3 mg/L and post-treatment (median: 0.1 mg/L; range: 0.1 to 9.4 mg/L; n = 31; p-value = 0.0281) cTLI concentrations.

5.7 Serum S100A12 concentrations

Serum S100A12 results could not be obtained for 1 patient in the 12 mg/kg/d group and 2 patients in the 24 mg/kg/d group on account of logistic restraints regarding assay availability. Serum S100A12 concentrations decreased significantly between pre-treatment (median: 160 µg/L; range: 52 to 1327 µg/L) and post-treatment (median: 88 µg/L; range: 43 to 449 µg/L) concentrations in the 12 mg/kg/d group (n=11; p=0.0010). However, serum S100A12 concentrations did not change significantly between pre-treatment (median: 147 µg/L; range: 41 to 973µg/L) and post-treatment (median: 148 µg/L; range: 58 to 746µg/L) concentrations in the 24 mg/kg/d group (n=17; p=0.8176) or between pre-treatment (median: 154 µg/L; range: 41 to 1327 µg/L) and post-treatment (median: 133 µg/L; range: 43 to 746 µg/L) concentrations for all treated dogs combined (n=28; p=0.1438).

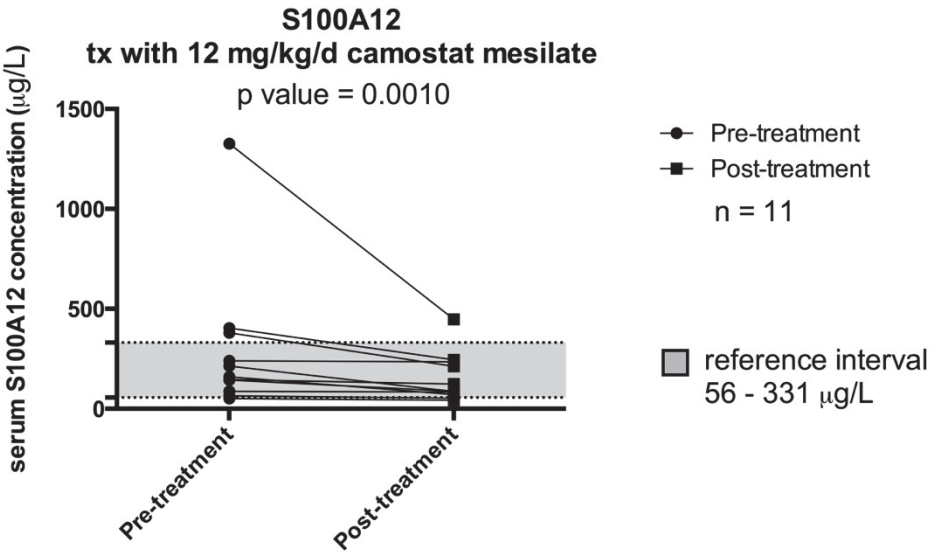

Figure 19 Serum S100A12 concentrations tx 12 mg/kg/d This figure shows serum S100A12 concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare the values before and after treatment. There was a significant decrease between pre-treatment (median: 160 µg/L; range: 52 to 1327 µg/L) and post-treatment (median: 88 µg/L; range: 43 to 449 µg/L; n = 11; p-value = 0.001) S100A12 concentrations.

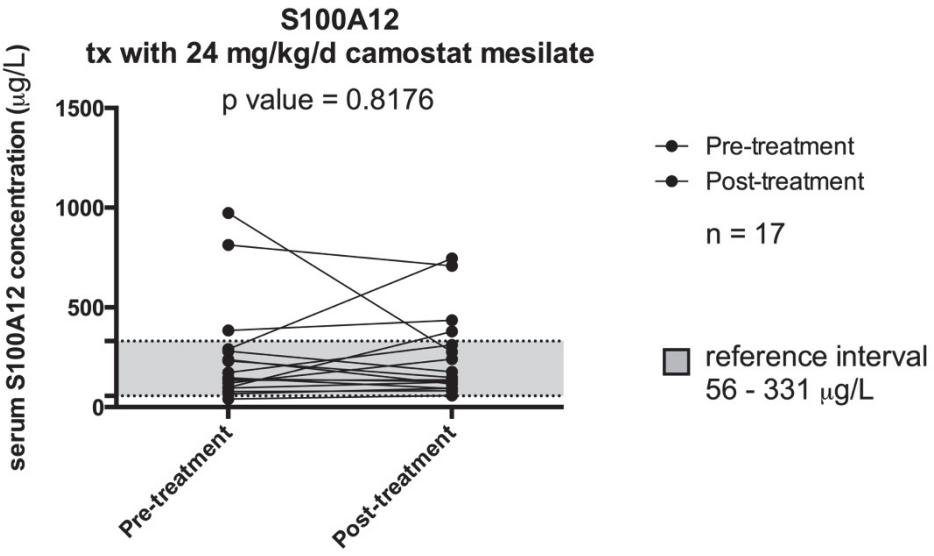

Figure 20 Serum S100A12 concentrations tx 24 mg/kg/d This figure shows serum S100A12 concentrations in dogs with suspected chronic pancreatitis before and after treatment with 24 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare the values before and after treatment. There was no significant difference between pre-treatment (median: 147 µg/L; range: 41 to 973µg/L) and post-treatment (median: 148 µg/L; range: 58 to 746µg/L L; n = 17; p-value = 0.8176) S100A12 concentrations.

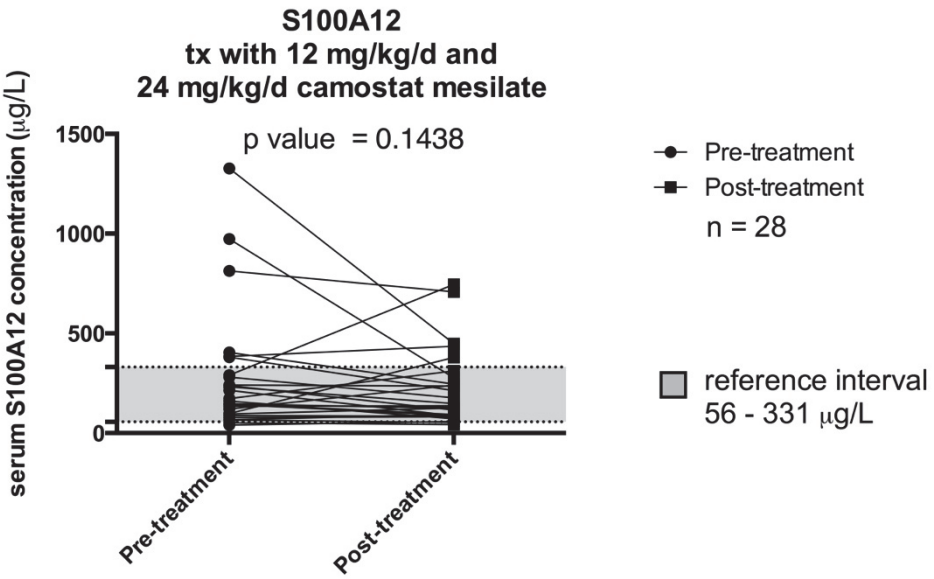

Figure 21 Serum S100A12 concentrations tx 12 mg/kg/d and 24 mg/kg/d combined This figure shows serum S100A12 concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg and 24 mg/kg of camostat mesilate/d. A Wilcoxon matched-pair signed rank test was used to compare the values before and after treatment. There was no significant difference between pre-treatment (median: 154 µg/L; range: 41 to 1327 µg/L) and post-treatment (median: 133 µg/L; range: 43 to 746 µg/L; n = 28; p-value = 0.1438) S100A12 concentrations.

5.8 Serum TGF- β1 concentrations

Serum TGF-β1 concentrations did not change significantly between pre-treatment (mean: 45 ng/mL; SD: ± 13 ng/mL) and post-treatment (mean: 43 ng/mL, SD: ± 8 ng/mL) values in the 12 mg/kg/d group (n=12; p-value=0.3679) or between pre-treatment (median: 39 ng/mL; range: 20 - 99 ng/mL) and post-treatment (median: 40 ng/mL; range: 25 - 64 ng/mL) concentrations in the 24 mg/kg/d group (n=19; p-value=0.1956). There was a trend towards a decrease in post-treatment serum TGF-β1 concentrations but no statistically significant difference between pre-treatment (median: 48 ng/mL; range: 17 - 99 ng/mL) and post-treatment (median: 41 ng/mL; range: 25 - 64 ng/mL) concentrations for all treated dogs combined (n=31; p=0.0695).

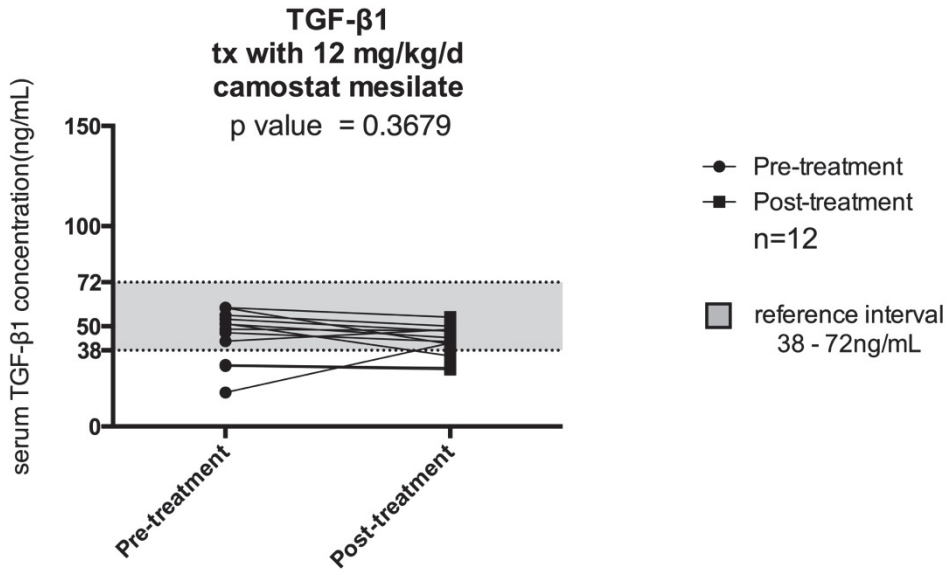

Figure 22 Serum concentrations TGF- β 1 tx 12 mg/kg/d This figure shows serum TGF-β1 concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg of camostat mesilate/d. A paired t-test was used to compare values before and after treatment. There was no significant difference between pre-treatment (mean: 45 ng/mL; SD: ± 13 ng/mL) and post-treatment (mean: 43 ng/mL, SD: ± 8 ng/mL; n = 12; p-value = 0.3679) TGF- β 1 concentrations.

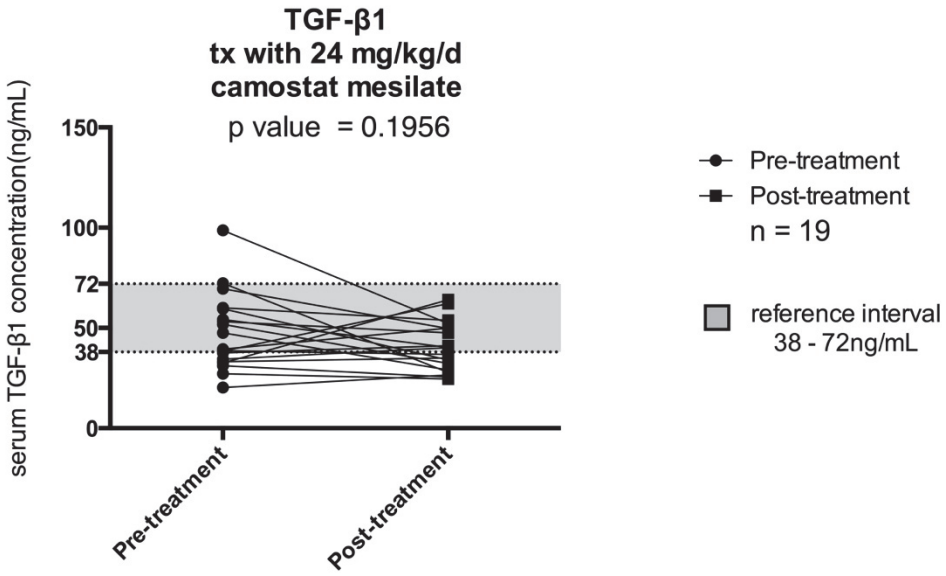

Figure 23 Serum concentrations TGF- β 1 tx 24 mg/kg/d This figure shows serum TGF- β 1 concentrations in dogs with suspected chronic pancreatitis before and after treatment with 24 mg/kg of camostat mesilate/d. A paired Wilcoxon matched-pair signed rank test was used to compare values before and after treatment. There was no significant difference between pre-treatment (median: 39 ng/mL; range: 20 - 99 ng/mL) and post-treatment (median: 40 ng/mL; range: 25 - 64 ng/mL; n=19; p-value = 0.1956) TGF- β1 concentrations.

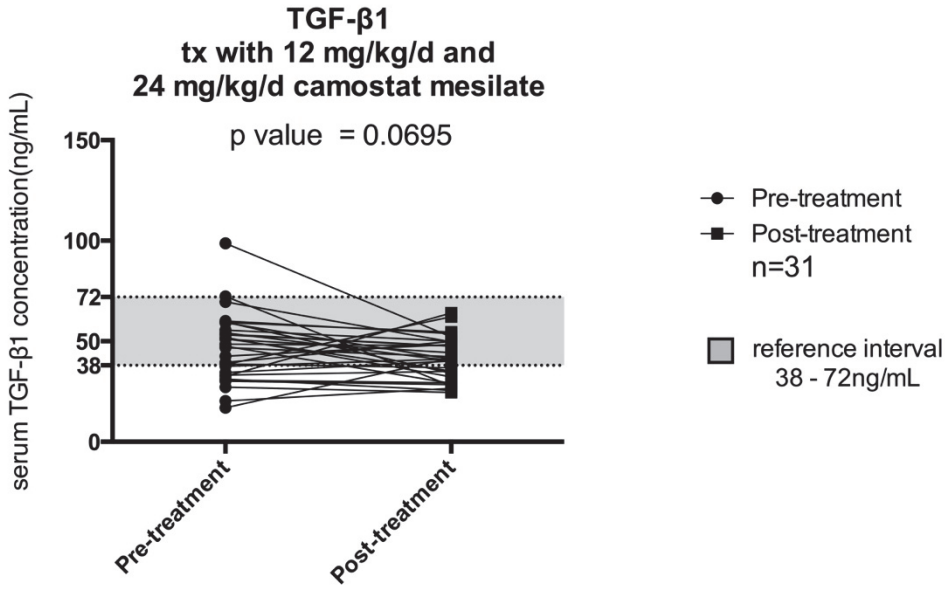

Figure 24 Serum concentrations TGF- β 1 tx 12 mg/kg/d and 24 mg/kg/d combined This figure shows serum TGF- β 1 concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg and 24 mg/kg of camostat mesilate/d. A paired Wilcoxon matched-pair signed rank test was used to compare values before and after treatment. There was no significant difference between pre-treatment (median: 48 ng/mL; range: 17 - 99 ng/mL) and post-treatment (median: 41 ng/mL; range: 25 - 64 ng/mL; n=31; p-value = 0.0695) TGF- β 1 concentrations.

5.9 Serum cobalamin concentrations

Serum cobalamin concentrations decreased significantly between pre-treatment (mean: 868 ng/L; SD: ± 108ng/L) and post-treatment (mean: 695 ng/L; SD: ± 223 ng/L) concentrations in the 12 mg/kg/d group (n=12; p=0.0363). Serum cobalamin concentrations decreased significantly between pre-treatment (mean: 749 ng/L; SD: ± 229 ng/L) and post-treatment (mean: 641 ng/L; SD: ± 252 ng/L) concentrations in the 24 mg/kg/d group (n=19; p=0.045), but no statistically significant change between pre-treatment (mean: 778 ng/L; SD: ± 221 ng/L) and post-treatment (mean: 686 ng/L; SD: ± 226 ng/L) concentrations was identified for this group (n=17; p=0.109) after removal of patients that had received cobalamin supplementation (n=2). Serum cobalamin concentrations decreased significantly between pre-treatment (mean: 843 ng/L; SD: ± 198 ng/L) and post-treatment (mean: 681 ng/L; SD: ± 239 ng/L) concentrations for all treated dogs combined (n=31; p=0.003), and between pre-treatment (median: 855 ng/L; range: 262 to 1001 ng/L) and post-treatment (median: 681 ng/L; range: 239 to 1001 ng/L) concentrations for all treated dogs combined when patients that had received cobalamin supplementation (n=5) and patients that possibly received cobalamin supplementation (n=3) had been removed (n=23, p=0.008). There were no significant correlations between treatment duration and differences between pre-treatment and post-treatment serum cobalamin concentrations for the 24 mg/kg/d group (n=17; ρ=- 0.4; p-value: 0.1) or all treated dogs combined (n=23; ρ= -0.13; p-value: 0.56).

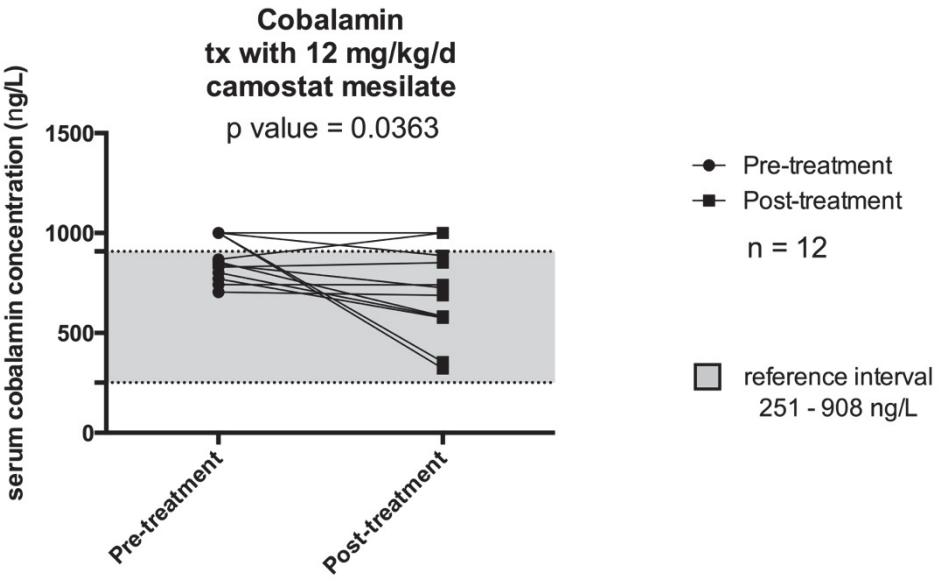

Figure 25 Serum cobalamin concentration for the 12 mg/kg/d treatment group. This figure shows serum cobalamin concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg of camostat mesilate/d. A paired t-test was used to compare values before and after treatment. There was a significant decrease between pre-treatment (mean: 868 ng/L; SD: ± 108ng/L) and post-treatment (mean: 695 ng/L; SD: ± 223 ng/L; n=12; p-value=0.0363) serum cobalamin concentrations.

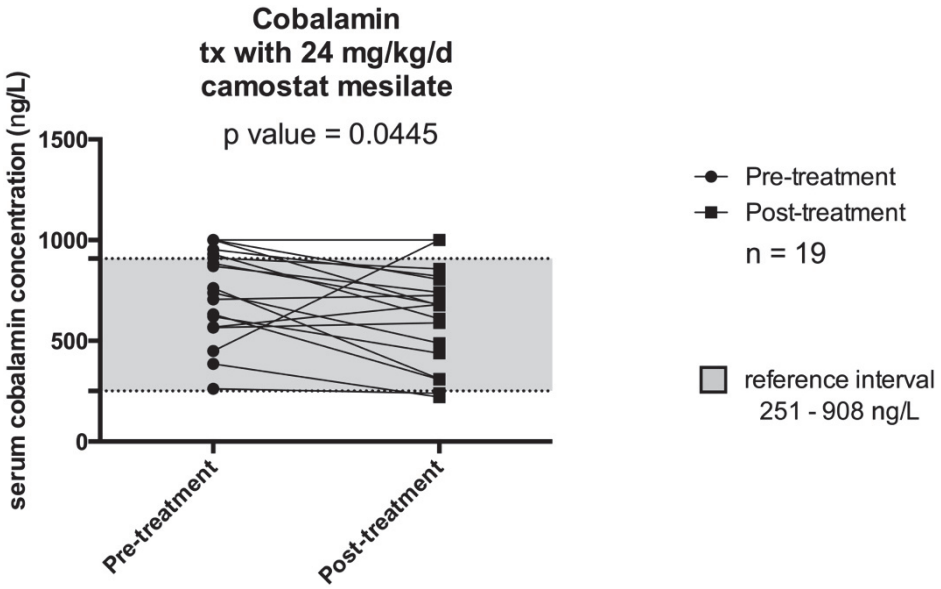

Figure 26 Serum cobalamin concentrations for the 24 mg/kg/d treatment group. This figure shows serum cobalamin concentrations in dogs with suspected chronic pancreatitis before and after treatment with 24 mg/kg of camostat mesilate/d. A paired t-test was used to compare values before and after treatment. There was a significant decrease post-treatment (mean: 641 ng/L; SD: ± 252 ng/L) when compared to pre-treatment (mean: 749 ng/L; SD: ± 229 ng/L; n=19; p-value=0.0445) serum cobalamin concentrations.

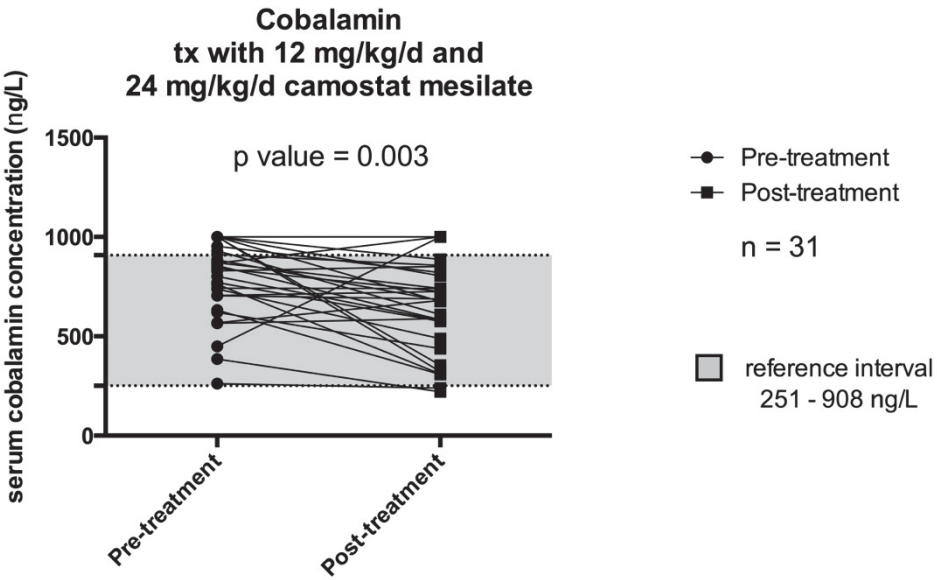

Figure 27 Serum cobalamin concentrations for all treated dogs This figure shows serum cobalamin concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg or 24 mg/kg/d of camostat mesilate. A paired t-test was used to compare values before and after treatment. There was a significant difference between pre-treatment (mean: 843 ng/L; SD: ± 198 ng/L) and post-treatment (mean: 681 ng/L; SD: ± 239 ng/L; n=31; p-value=0.003) serum cobalamin concentrations.

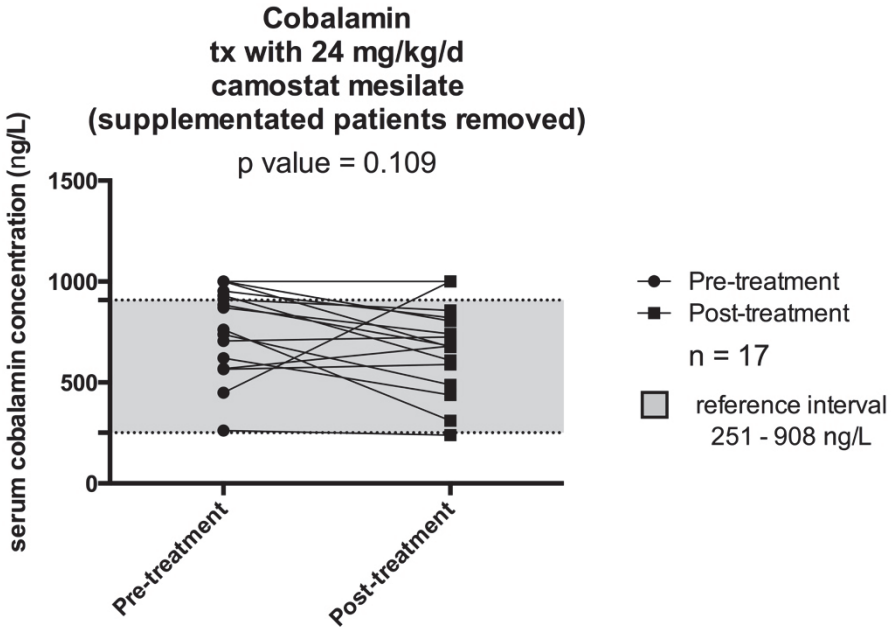

Figure 28 Serum cobalamin concentrations for the 24 mg/kg/d treatment group after supplemented patients had been removed This figure shows serum cobalamin concentrations in dogs with suspected chronic pancreatitis before and after treatment with 24 mg/kg/d of camostat mesilate after patients that had received cobalamin supplementations (n=2) had been removed. A paired t-test was used to compare values before and after treatment. There was no significant difference between pre-treatment (mean: 778 ng/L; SD: ± 221 ng/L) and post-treatment (mean: 686 ng/L; SD: ± 226 ng/L; n=17; p=0.109) serum cobalamin concentrations.

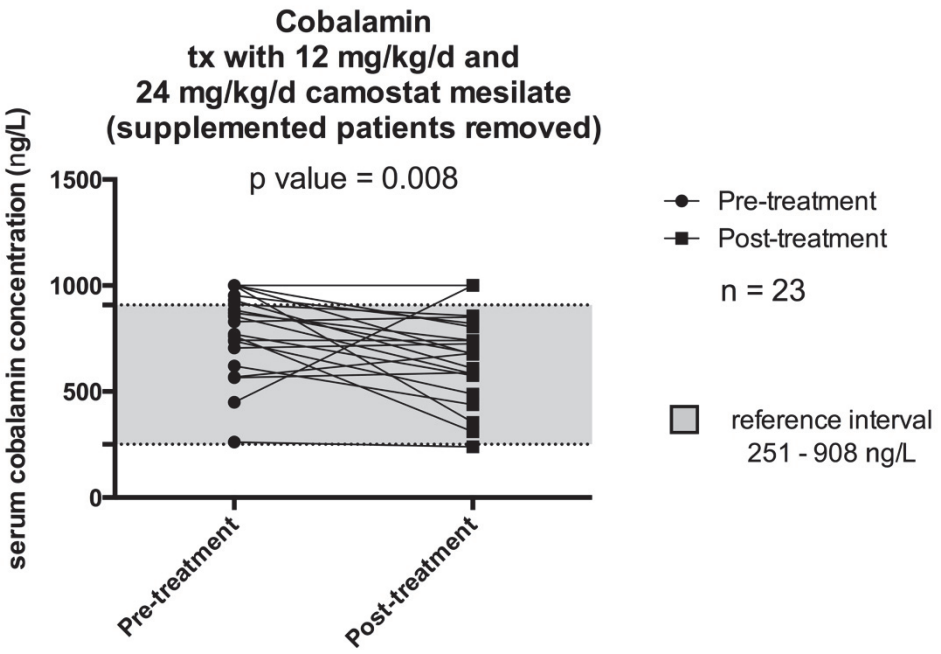

Figure 29 Serum cobalamin concentrations for all treated dogs after –supplemented patients had been removed This figure shows serum cobalamin concentrations in dogs with suspected chronic pancreatitis before and after treatment with 12 mg/kg or 24 mg/kg/d of camostat mesilate after patients that had received cobalamin supplementations (n=3) and patients that possibly received cobalamnin concentrations (n=3) had been removed. A Wilcoxon matched-pair signed rank test was used to compare values before and after treatment. There was a significant difference between pre-treatment (median: 855 ng/L; range: 262 to 1001 ng/L) and post-treatment (median: 681 ng/L; range: 239 to 1001 ng/L; n=23; p=0.008) serum cobalamin concentrations.

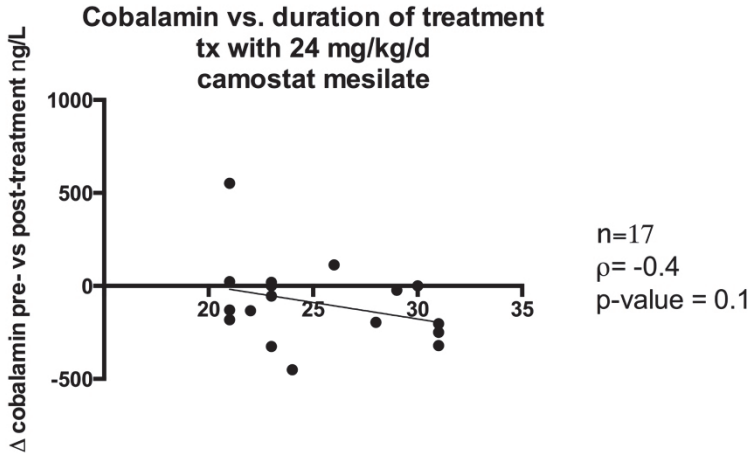

Figure 30 Duration of treatment and differences in serum cobalamin between pre-and post-treatment tx 24 mg/kg/d This Figure shows the correlation between the duration of treatment and differences in serum cobalamin concentrations in dogs with suspected chronic pancreatitis before and after treatment with 24 mg/kg of camostat mesilate/d. Spearman nonparametric correlation was used to measure correlations of treatment duration and cobalamin concentration. There was no correlation between treatment length and decreases in serum cPLI concentration (n=17; r_s= 0.195; p-value: 0.1).

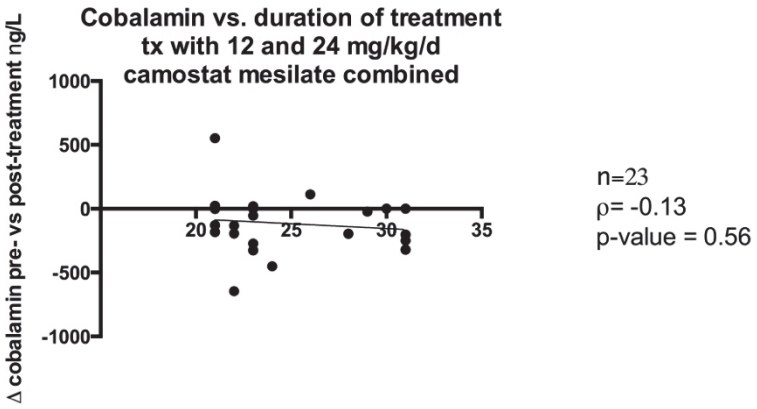

Figure 30 Duration of treatment and differences in serum cobalamin concentrations between pre-and post-treatment for all treated dogs This Figure shows the correlation between the duration of treatment and differences in serum cobalamin concentrations in dogs with suspected chronic pancreatitis before and after treatment with 24 mg/kg of camostat mesilate/d. Spearman nonparametric correlation was used to measure correlations of treatment duration and cobalamin concentration. There was no correlation between treatment length and decreases in serum cobalamin concentration (n=23; r_s= -0.13; p-value: 0.56).

5.10 Serum folate concentrations

Serum folate concentrations did not change statistically significantly in either treatment group or for all treated dogs combined.

5.11 Serum biochemistry and complete blood count

No statistically significant differences were observed for pre- and post-treatment CBC or serum biochemistry profile parameters (data not shown). However, one dog in the 24 mg/kg/d treatment group presented with a 35-fold increase in serum GGT activity and a 6-fold increase in AST activity after treatment (AST: 36 U/L to 178 U/L; RI: 12 – 48; GGT: 5 U/L to 177 U/L; RI: 0 – 12 U/L). No follow-up data were available to investigate whether or not these findings normalized after discontinuation of therapy.

5.12 Follow-up questionnaires

Quality of life had improved in 4/12 dogs (33%), not changed in 4/12 dogs (33%), and declined in 0/12 dogs (0%) in response to treatment with 12 mg/kg/d. Four veterinarians (33%) were unavailable for follow-up questioning. Quality of life had improved in 13/19 dogs (68%), not changed in (6/19) dogs (32%), and declined 0/19 dogs (0%) in response to treatment with 24 mg/kg/d. All veterinarians were available for follow-up questioning in this group. For all treated dogs, an improvement in quality of life was reported for 17/31 dogs (55%), no change was reported for 10/31 dogs (32%), and a decline in quality of life had been reported for 0/31 dogs (0%). 4 out of 31 veterinarians (13%) were unavailable for follow-up questioning.

6 DISCUSSION

This is the first trial assessing the efficacy of camostat mesilate in dogs with naturally-occurring suspected chronic pancreatitis and literature or previous studies offer no links between the drug and the investigated core markers. It is important to recognize these and other limitations, and the implications they have on any intervention-related conclusions.

6.1 Study design

One such limitation is inherent to the chosen study design. Clinical trials are usually conducted in 4 different phases, or steps, wherein each step utilizes an increasing sample population in order to answer a separate and specific research question. Phases I and II routinely involve smaller subject populations than subsequent phases. Their goal is to evaluate safety, establish dosage ranges, identify adverse effects, and study efficacy before larger target populations are exposed. Ideally, such studies include a treatment and a control group, to which study participants are assigned at random. In addition, investigators and participants are blinded as to which individual receives the intervention or a placebo. Inclusion of a control group, a placebo and double blinding significantly reduces spurious causality and bias. Double blinded randomized controlled trials are thus considered the gold standard model of evidence-based medicine. However, logistical, economical, and sometimes ethical restraints may require a different approach.

In this particular case, another study design was considered more appropriate, resulting in a mixture of the first two phases of a classical clinical trial, with the additional research goal of answering questions regarding fundamental properties of CM. This decision was made firstly, because only a very limited number of approximately 30 dogs could be enrolled. Secondly, because the appropriate dosage was unknown, and thirdly because efficacy of CM for the treatment of canine CP had not previously been evaluated. Inclusion of a control group was not feasible due to both ethical and financial considerations. This study design merely serves to examine the effects of a treatment or intervention on a specific population. No definitive causal inferences as to the effects of a specific intervention, i.e., treatment with camostat mesilate, can be deduced from the results of this study type. However, it does allow conclusions about the effects of CM on the specific patient population of this research effort. Therefore, scope and design of this study limit the level of conviction with which any assertions can be made with regards to CM treatment of dogs with CP outside of this particular treatment group. Future study designs that aim to investigate the efficacy of CM for the treatment of canine pancreatitis will need to be tailored accordingly, i.e., contain a control group and feature double blinding, placebo treatments, and ideally, randomization of group affiliation.

Another limitation of this study is the lack of pre- and post-treatment pancreatic biopsies and histopathologic confirmation of CP. Thus, based on a history clinical signs that are compatible with a diagnosis of pancreatitis in conjunction with a history of elevations in serum cPLI concentrations, disease in all enrolled dogs was classified as suspected chronic pancreatitis. Due to this lack of histopathology as part of this study, the evaluation of CM's effect on acinar cell damage, fibrosis, or inflammation relies solely on surrogate markers and remains, at best, tentative. It is therefore impossible to evaluate if and how variations in laboratory parameters translate into physical changes.

6.2 Dosage range

In people, a dose of 200 mg given three times a day for a total of 600 mg/d has been established in various studies (Ishii, 1980; Ishii, 1984). This translates to approximately 10 mg/kg/d. Preliminary literature investigation uncovered only one article that reported effects of varying CM dosages in dogs. These results, stemming from a sub-acute toxicity study (Matsuoka, 1980), provided a general idea as to the extent of CM's safety range but left many questions unanswered. Firstly, only a small and homogeneous sample population of Beagle

dogs had been used in this particular study. This contrasts with the very heterogeneous patient pool to be expected in a study conducted with privately owned animals. Secondly, the lowest dose that was well tolerated by all subjects and caused no adverse effects (30 mg/kg/d) is roughly one third of the lowest dose that induced vomiting (100 mg/kg/d; 50 mg/kg q 12 hrs) in a number of experimental dogs. Effects of a large dosage range of CM are therefore unknown. Thirdly, all Beagle dogs in the sub-acute toxicity study were healthy and treatment was considerably shorter than the 26±5 days chosen for this study. These considerations resulted in conservative dosages of 12mg/kg/d and 24 mg/kg/d and two differently sized treatment groups. Once 12 patients had completed the study, data were analyzed and it was determined that 12 mg/kg/d elicited neither a positive response with respect to core measurements, nor any adverse drug effects. An increase to 24mg/kg was proposed and the animal care and use protocol was amended accordingly. These changes were reviewed and approved by the Texas A&M Institutional Animal Care and Use Committee (Animal Use Protocol #2012-042).

12 mg/kg/d of CM failed to produce the same significant changes in biomarker concentrations as 24 mg/kg of CM/d did for some of the biomarkers evaluated. Nevertheless, the higher dosage did not elicit any major or more pronounced adverse effects than CM at the lower one. Considering our very limited knowledge of CM's dose-effect relationship in dogs, this is an interesting insight and gives rise to the question what the "correct" dosage of CM for the treatment of chronic pancreatitis in dogs may be. Future studies are necessary to elucidate whether a dose increase is safe and efficacious.

6.3 Signalment

Some of the breeds reported to have a predisposition for chronic pancreatitis, including Cavalier King Charles Spaniels, Cocker Spaniels, Boxers, and Miniature Schnauzers, were represented in the trial population. Although Dachshunds (6/31) and mixed breed dogs (6/31) were the prevailing breeds, 4 Miniature Schnauzers, 2 Cavalier King Charles Spaniels, and 1 Boxer were among the trial population. Miniature Schnauzers are among the breeds presented more often for CP than dogs of other breeds and the third most prevalent breed in this study, but none presented with the increased serum triglyceride concentrations at the time of enrollment, which has been reported to be associated with increased risk for pancreatitis (Xenoulis et al., 2010). However, one Miniature Schnauzer presented with a serum

triglyceride concentration of 1,109 mg/dL post-treatment. The question whether or not any of these dogs had severely increased serum triglyceride concentrations previously, but presented with only mildly increased ones at the time of the pre-treatment workup could not be determined.

The majority of patients were middle-aged (5-8 a; 7/31) or older (>9 a; 21/31), with an overall average age of 10.3 yrs. A similar distribution can be found in the GI Lab database for dogs with a cPLI > 400 µg /L (**Table 6**) and seems to confirm findings from a previous study which identifies this to be the typical age for dogs to presented with CP (Watson et al., 2007) and elevated serum cPLI concentrations.

Table 6: Age distribution GI Lab 2014 cPLI > 400 µg/L, CM study

GI Lab database 2014		CM study	
Age	**Number**	**Age**	**Number**
Young	388	Young	3
Middle-aged	837	Middle-aged	7
Old	2162	Old	21
Total: 3387	3387		31

Table 6 Age distribution GI Lab database accessions with a cPLI result > 400 µg/L from January 2014 – January 2015 (n=3387); CM study age distribution for all dogs treated.

Table 7: Breed distribution GI Lab 2014 cPLI >400µg/L, AKC 2013, CM study

GI Lab database 2014		American Kennel Club 2013		CM study	
Breed	**Rank**	**Breed**	**Rank**	**Breed**	Rank
Mixed breeds (n=646)	1			Mixed breeds (n=6)	1
Dachshunds (n=138)	5	Dachshunds	10	Dachshunds (n=6)	1
Miniature Schnauzers (n=131)	6	Miniature Schnauzers	17	Miniature Schnauzers (n=3)	2
Cavalier K Charles Spaniels (n=85)	9	Cavalier K Charles Spaniels	18	Cavalier K Charles Spaniels (n=2)	3
Total: 3387				Total: 31	

Table 7 Breed distribution of GI Lab database accession with a cPLI result > 400 µg/L between January 2014 and January 2015 (n=3387); American Kennel Club dog registration statistics for 2013 (American Kennel Club, 2014); CM study breed distribution for all dogs treated.

Concerning dog breeds, it is interesting to compare a) the breed and age distribution of dogs in the GI Lab database with a cPLI concentration >400 µg/L and b) the most common breeds registered with the American Kennel Club with c) those breeds found in this study (**Table 7**). The Dachshunds, Miniature Schnauzers, and Cavalier King Charles Spaniels that rank highest in this study are also found more frequently among the dogs in the GI lab database, when compared to the breeds most commonly registered with the American Kennel Club. Even when the major limitation of this comparison is considered - only a fraction of these 3387 dogs were diagnosed with CP - the particular breed distribution in this study still relies on a low sample number. These results therefore neither disprove previous findings concerning breed prevalence for CP, nor suffice to identify other breeds that may be at increased risk.

6.4 Serum cPLI – marker for acinar cell damage

When evaluating the efficacy of a new treatment, biomarkers are commonly used as surrogate markers for a clinically meaningful endpoint and as a means of predicting the effect of an intervention (Katz, 2004). These markers usually correlate with the progression of the disease in question and their use is uncontroversial from a regulatory standpoint if they are reasonably likely to predict a clinical benefit (Katz, 2004). The effects of CM on cPLI concentrations have not previously been investigated but a number of considerations make the measurement of cPLI in serum a suitable surrogate marker for the evaluation of CM's efficacy in dogs with CP. Firstly acinar cell damage is directly reflected by serum concentrations of pancreatic lipase. Secondly, cPLI has been shown to be the most sensitive and specific diagnostic tool for both acute and chronic inflammatory lesions of the pancreas (Trivedi et al., 2011).

Decreases in serum cPLI concentrations were found in only 6/12 (50%) of dogs treated with the lower CM dosage. However, it is important to keep in mind that "no significance" does not automatically mean "no difference". Out of 19 patients that had received the higher dose 14 (63%) showed decreased serum cPLI concentration post-treatment. This statistically significant result potentially indicates ameliorating effects of CM on acinar cell damage when administered at a dose of 24 mg/kg/d. It is, at this point in time and without further investigation, impossible to extrapolate, whether a further dose increase beyond 24 mg/kg/d would correspond with further reductions in serum cPLI concentrations.

Correlations between treatment duration and changes in cPLI lacked significance. Inclusion of a control group, as well as implementing a larger sample size, with repeated measurements over a longer period of time would be possible ways to answer the question of whether or not a correlation exists between serum cPLI concentrations and treatment duration.

No data concerning additional treatments were collected. Anti-emetics, analgesics, and/or other medications were likely administered in some cases. Yet, to the author's knowledge attenuation of acinar cell damage and the associated decrease of cPLI concentration have not previously been linked to any of these treatments. As per instructions to participating veterinarians and patient owners alike, individual diets remained the same throughout the study period but were not recorded. Low-fat prescription diets are a recommended and now common supportive measure, based on the proposed involvement of elevated serum triglycerides in the etiology of CP. But whether or not they lead to any changes in serum cPLI concentrations is, to date, uncertain and no evidence for this can be found in literature.

6.5 Quality of life

No post-treatment follow up questionnaires could be obtained for one third of the dogs in the 12 mg/kg/d treatment group, lowering the number of QoL evaluations in this group to 8. Indeed, improvements in QOL appeared to be more pronounced in the higher dosage group, but the low sample number in the 12 mg/kg/d group precludes comparisons between the two. Interestingly, if one removes the 4 patients for whom no follow-up questionnaires could be obtained from the gross patient pool, an increase in QOL was reported for 17 out of 27 dogs (63%), 13 of which also showed a decrease in serum cPLI concentration. As for the 24 mg/kg/d treatment group alone, an increase in QOL was reported for 13/19 (68%) dogs, 10 of which also showed a post-treatment serum cPLI decrease.

Putting changes in QOL and cPLI into perspective, one might conclude the two are inversely correlated. Given, the majority of patients with a decrease in cPLI concentration did experience an increase in QOL. However, 3 patients with a reported increase in QOL showed an increase in post-treatment serum cPLI concentrations. Conversely, QOL did not change in 4 dogs that showed decreased serum cPLI concentration post-treatment. If each possible QOL outcome is assigned a number (no change = 0, improvement = 1, decline = -1), it is possible to statistically analyze the development of QOL when compared to a baseline for which a

zero-value (0) is assumed. The resulting data show a statistically significant improvement of QOL in the 24 mg/kg/d group (n=19; p=0.002) as well as the combined patient pool (n=27; p=0.001; patients without follow-up questionnaire removed). Based on these results, the entirety of available post-treatment questionnaires and personal communication with participating veterinarians and pet owners, it appears CM had an overall beneficial effect on the quality of life of patients enrolled in this study.

Nonetheless, it must be recognized that grading and evaluating quality of life in companion animals is difficult, not standardized, and highly subjective. Some fields of veterinary medicine benefit from established systems of disease classification that closely correlate with health-related QOL (Niessen et al., 2010; Freeman et al., 2012) and can be used to validate efficacy of treatment (Niessen et al., 2010). However, there are no clinical scoring/classification systems for canine CP, where clinical signs can be nondescript and often remain subclinical. This complicates measurement of QOL and should prompt caution when evaluating the collected data.

There is an argument to be made for extended questionnaires, especially with regards to concurrent treatments and individual diets. Because low-fat diets, anti-emetics, and analgesics play a large role as supportive measures in patients with CP, they are likely to alter QOL if initiated, discontinued, or changed during the treatment period. However, withholding treatments with the goal to homogenize trial conditions is ethically questionable, especially when relying on privately owned animals and owner compliance.

6.6 Adverse drug reactions

Also subject to personal bias are the reported clinical adverse drug reactions, which comprised increased flatulence (3/31), pruritus (1/31), and vomiting and diarrhea (1/31) in a total of 5/31 (16.1%) patients. Whether or not these signs are attributable to CM or constitute a byproduct of increased owner awareness and advertence once treatment with this new drug had begun is debatable.

The cause for the isolated increase in liver enzymes noted in one dog treated with 24 mg/kg is unclear. Remarkably, similar changes were neither associated with CM-treatment in literature, nor observed in other patients in this study. There was no indication for liver disease in this particular patient before treatment and no follow-up data were available to investigate whether or not these findings normalized after discontinuation of therapy. While

these findings could potentially be regarded as an adverse hepatic drug effect, they may very well not be reciprocal with treatment. With no evidence for any other side effects found during the evaluation of serum biochemistry and complete blood count results, one might conclude that CM is a safe medication for treatment of canine chronic pancreatitis. This appears to confirm observations made in people treated with CM for CP (Ishii, 1980; Ishii, 1984; Fukuda et al., 2009).

However, because this is the first time CM was used in this fashion in dogs, caution and further studies are warranted in order to investigate and establish the extent of CM's possible adverse effects.

6.7 Inflammatory markers CRP and S100A12

Although pronounced individual post-treatment reductions were observed for both biomarkers of inflammation, S100A12 reductions reached statistical significance only in the lower dosage group. Thus, the anti-inflammatory properties previously attributed to CM could not be conclusively reproduced in this study. Low admissible sample numbers for each biomarker inhibited effective judgment and assessment of CM's anti-inflammatory characteristics. Indeed, only few patients presented with increased pre-treatment serum concentrations of S100A12 (6/31) or CRP (5/31). Only 2 patients presented with increases in both marker molecules. This suggests that inflammatory reactions in the remaining study dogs lacked severity - and marker concentrations remained under the lower limit of detection - or remained inactive at the time of sampling.

It is unclear why significant S100A12 reductions were observed for the lower dose cohort, but not for the higher one - which contrasts with the trend for other markers, for example cPLI, used in this study. But these reductions are based on a very low overall numbers of patients with initially elevated marker concentrations for whom significant individual regression was observed. In general, S100A12 does not appear to be increased in many canine patients with CP and measurement of this marker for the purpose of diagnosing or monitoring chronic pancreatitis in dogs may not be useful. After all, S100A12 is almost exclusively expressed by granulocytes, whereas infiltrates associated with canine chronic pancreatitis are of mononuclear nature.

Results for CRP measurements are analogous to those of S100A12. On one hand few patients (n=5) showed increased serum concentrations of CRP pre-treatment. On the other

hand, individual decreases in some patients were marked. Because CRP expression is greatly up-regulated in response to various inflammatory processes and tissue damage (Black et al., 2004), it remains unclear whether or not the isolated CRP increases observed in some patients are truly attributeable to CP. Considering the low number of dogs with increased serum CRP and simultaneous cPLI concentrations indicative of acinar cell damage, it appears this biomarker is unsuited for monitoring and /or diagnosing CP in dogs. A more definitive evaluation of the effect of CM on systemic inflammatory markers might be possible if a larger number of patients with increased pre-treatment CRP and S100A12 serum concentrations were to be evaluated.

6.8 Fibrosis marker TGF-β 1

As mentioned previously, the lack of histopathological examination of pancreatic biopsies presents a major limitation to the accurate assessment of some of the tested parameters. This is especially true as it relates to pancreatic fibrosis, where no marker molecule has previously been described for dogs.

Post-treatment TGF-β1 reductions were insignificant for either treatment group (n=12; p-value=0.3679 and n=19; p-value=0.1956), but approached significance for the overall patient population (n=31; p=0.0695). The question whether or not prolonged treatment or a higher dosage would bolster this trend remains unanswered. Considering the majority of patients presented with pre-treatment TGF-β1 concentrations within or even below the reference interval, it is questionable if TGF-β1 concentrations accurately reflect fibrotic changes within the pancreatic parenchyma and can possibly serve as an appropriate biomarker for evaluating pancreatic fibrosis in dogs with naturally occurring suspected CP.

6.9 Serum cobalamin concentrations

Interestingly, follow-up questionnaires revealed a total of 5 dogs had received cobalamin supplementation just prior to enrollment into the study and it is important to bear in mind that a number of follow-up questionnaires remained unanswered. Removal of those dogs for which no follow-up data regarding this measure is available, in concert with confirmed supplementations results in the following patient numbers for which cobalamin concentrations can be considered “unaltered”: 6 dogs in the 12 mg/kg/d group and 17 dogs in

the 24 mg/kg/d group. Too few patients remained in the 12 mg/kg/d group for statistical analysis and changes between pre-treatment and post-treatment in the 24 mg/kg/d group were not statistically significantly different (n=17; p=0.109). However, there was a statistically significant difference for all treated dogs combined (n=23, p=0.008).

Because correlations between CM treatment duration and differences in pre- and post-treatment serum cobalamin concentrations of the adjusted patient population were statistically insignificant, no inference can be made as to the impact of CM on serum cobalamin concentrations over time. An increase in sample number and the addition of an untreated control group would be especially helpful, as serum cobalamin concentrations regress naturally over time in dogs that receive supplementation. The overall results and the fact that some of the patients presented with serum cobalamin concentrations below the detection limit further raises the question of whether or not more patients had received supplementation than was indicated to the investigator.

Despite the fact that 24 mg/kg/d of CM failed to produce a significant reduction once supplemented patients were removed, results for the overall patient population serve as an indicator for CM's suppressive effects on serum cobalamin concentrations. Compared to others, this particular measurement appears to be subject to a larger number of variables, introducing a significant spurious element that necessitates further studies including larger patient populations and more stringent follow-up guidelines with regard to cobalamin supplementation in order to determine whether or not any of these findings can be reproduced.

7 CONCLUSION

Treatment of dogs with camostat mesilate was associated with only very mild side effects, suggesting that this drug is generally safe for use in dogs. Also, in this study camostat mesilate at a dose of 24 mg/kg/d led to a subjective improvement of quality of life and a significant decrease in serum cPLI concentrations in dogs with suspected chronic pancreatitis, suggesting an ameliorating effect on acinar cell damage of this new drug at this dosage. However, CM did not lead to any significant changes in serum concentrations of CRP, S100A12, or TGF-β1, even though marked decreases were observed in individual patients. There is an indication CM causes a decrease in serum cobalamin concentrations. These findings would suggest that larger controlled studies in dogs with chronic pancreatitis are warranted.

8 SUMMARY

Chronic pancreatitis is an inflammatory condition of the pancreas characterized by atrophy, fibrosis, and loss of function. Due to a lack of designated pharmaceutical agents, treatment of canine chronic pancreatitis is usually limited to supportive care. There are reports from Japan about the use of a protease inhibitor, camostat mesilate, for the treatment of chronic pancreatitis in humans, but no controlled studies about the efficacy of camostat mesilate for the treatment of chronic pancreatitis in dogs are available. Therefore, the primary goals of this study were to investigate the efficacy of camostat mesilate in dogs with suspected chronic pancreatitis as well as assess its effect on acinar cell damage by measuring serum concentrations of canine pancreas lipase immunoreactivity (cPLI). Also, the effect of the compound on systemic inflammation was quantified by measurement of serum concentrations of canine C-reactive protein and S100A12. Finally, the effect of camostat mesilate on fibrosis was assessed by measurement of serum concentrations of transforming growth factor (TGF)-β1. Thirty-one dogs with suspected chronic pancreatitis based on clinical signs and repeated measurements of an increased cPLI concentration above the cut-off value for the diagnosis of pancreatitis (≥ 400 µg/L) received a dose of 4 or 8mg/kg camostat mesilate q 8 h (12 or 24 mg/kg q 24 h) over a period of 26 ± 5 days.

The evaluation of follow-up questionnaires from referring veterinarians and owners suggested improvement in quality of life in some patients. Only mild side effects were

observed. Dogs given a daily camostat mesilate dose of 24 mg/kg/d orally showed a statistically significant decrease in serum cPLI concentrations between pre-treatment (median: 847 µg/L; range: 414 to 2,024 µg/L) and post-treatment (median: 520; range: 193 to 2,580 µg/L; n=19; p=0.016), indicating attenuation of acinar cell damage. Anti-inflammatory effects were comprised of a significant decrease of serum S100A12 concentrations in dogs treated with 12 mg/kg/d camostat mesilate between pre-treatment (median: 160 µg/L; range: 52 to 1327 µg/L) and post-treatment (median: 88 µg/L; range: 43 to 449 µg/L; n=11; p=0.0010). Serum cobalamin concentrations differed significantly between pre-treatment (mean: 868 ng/L; SD: ± 108ng/L) and post-treatment (mean: 695 ng/L; SD: ± 223 ng/L) concentrations in the 12 mg/kg/d group (n=12; p=0.0363) and also between pre-treatment (mean: 749 ng/L; SD: ± 229 ng/L) and post-treatment (mean: 641 ng/L; SD: ± 252 ng/L) concentrations in the 24 mg/kg/d group (n=19; p=0.045). However, no statistically significant changes in TGF-β concentrations, C-reactive protein, parameters of serum biochemistry, or complete blood counts were observed with treatment. This study would suggest a potentially beneficial role of camostat mesilate in dogs with chronic pancreatitis; however, case-control studies are needed to confirm these findings.

9 ZUSAMMENFASSUNG

AUSWIRKUNGEN EINES SYNTHETISCHEN SERINPROTEASEHEMMERS, CAMOSTAT MESILATE (FOY-305), AUF MARKER VON AZINARZELLSCHADEN, ENTZUENDUNG, UND FIBROSE BEI HUNDEN MIT VERMUTETER NATUERLICH VORKOMMENDER CHRONISCHER BAUCHSPEICHELDRUESENENTZÜNDUNG

Charakteristische Merkmale der chronischen Bauchspeicheldrüsenentzündung sind Atrophie, Fibrose und Funktionsverlust des Pankreasparenchyms. Da spezifische Therapeutika zur Behandlung der chronischen Pankreatitis beim Hund derzeit nicht zur Verfügung stehen, zielt die Behandlung in der Regel darauf ab, bestehende Symptome zu lindern und ein progressives Voranschreiten der Erkrankung zu verlangsamen. Berichte aus Japan beschreiben die Behandlung der chronischen Bauchspeicheldrüsenentzündung des Menschen mit dem Proteaseinhibitor Camostat Mesilate. Jedoch existiert keine Literatur, welche die Wirksamkeit bei Hunden im Rahmen eines Kontrollversuches dokumentiert.

Daraus folgend bestanden die Ziele dieser Studie darin, Camostat Mesilate einerseits hinsichtlich dessen Wirkung auf die Behandlung von Hunden mit vermuteter chronischer Bauchspeicheldrüsenentzündung zu untersuchen, und andererseits Auswirkungen der Therapie auf geschädigte Azinuszellen mittels der Auswirkung auf die Konzentration der caninen Pankreaslipaseimmunreaktivität (cPLI) im Serum zu erfassen.

Weiterhin wurden die Auswirkungen des Präparates auf Serumkonzentrationen der Entzündungsmarker C-reaktives Protein und S100A12-Protein, sowie des Fibrosemarkers TGF-β1 ermittelt.

Einunddreißig Hunde, welche aufgrund einer typischen klinischen Manifestation und wiederholt erhöhten cPLI Konzentrationen über dem Schwellenwert von 400 µg/L vermutlich an chronischer Pankreatitis erkrankt waren, wurden über eine Zeitraum von 26 ± 5 Tagen dreimal täglich mit 4 oder 8 mg/kg KGW (12 oder 24 mg/kg KGW pro Tag) mit Camostat Mesilate behandelt.

Die Auswertung der von den Tierärzten behandelter Hunde ausgefüllten Fragebögen deutet darauf hin, dass bei manchen Patienten eine Verbesserung der Lebensqualität beobachtet werden konnte. Lediglich geringfügige unerwünschte Arzneimittelwirkungen wurden verzeichnet. Bei jenen Hunden, die mit einer täglichen Dosis von 24 mg/kg KGW behandelt wurden, sank die cPLI Serumkonzentration statistisch signifikant zwischen prä- (Median: 847 µg/L; Spannweite: 414 bis 2,024 µg/L) und post-Therapie (Median: 520; Spannweite: 193 bis 2,580 µg/L; n=19; p=0.016). Dies deutet auf eine Minderung der Schädigung von Azinuszellen des Pankreasparenchyms hin.

Eine antiinflammatorische Arzneimittelwirkung konnte lediglich für S100A12, dessen Serumkonzentration in der mit 12 mg/kg KGW pro Tag behandelten Gruppe zwischen prä- (Median: 160 µg/L; Spannweite: 52 bis 1327 µg/L) und post-Therapie (Median: 88 µg/L; Spannweite: 43 bis 449 µg/L; n=11; p=0.0010) signifikant abnahm, beobachtet werden. Serumkonzentrationen von Cobalamin waren signifikant verändert zwischen prä- (Mittel: 868 ng/L; Standardabweichung: ± 108ng/L) und post-Therapie (Mittel: 695 ng/L; Standardabweichung: ± 223 ng/L; n=12; p=0.0363) in der mit 12 mg/kg KGW pro Tag und zwischen prä- (Mittel: 749 ng/L; Standardabweichung: ± 229 ng/L) und post-Therapie (Mittel: 641 ng/L; Standardabweichung: ± 252 ng/L; n=19; p=0.045) in der mit 24 mg/kg KGW pro Tag behandelten Gruppe. Nach der Behandlung wurden jedoch keine statistisch signifikanten Veränderungen der Serumkonzentrationen von TGF- β1 und C-reaktivem Protein, sowie Parameter von klinischer Chemie und großem Blutbild beobachtet.

Das Gesamtergebnis dieser Studie deutet auf eine potentiell positive Wirkung von Camostat Mesilate bei der Behandlung der chronischen Bauchspeicheldrüsenentzündung des Hundes hin; allerdings sind weitergehende Studien notwendig, um diese Schlussfolgerung zu bestätigen.

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11 APPENDIX

Follow-up questionnaire

Evaluation of a new medication (camostat mesilate) for the treatment of chronic pancreatitis in dogs

Patient:

Camostat mesilate shipped / trial started on:

1. Was cobalamin supplemented just prior to the study? Yes ☐ No ☐
   a. If you answered "Yes", when and how was cobalamin supplemented and at what dose?
2. How would you describe your patient's quality of life during the study?
   a. No change ☐
   b. Improvement ☐
   c. Decline ☐
   d. Can't say ☐
3. What was your patient's body weight at the end / 4 weeks after the beginning of the study?
   Kg_____ date_____

Figure 31: Veterinarian follow-up questionnaire

date	attitude	appetite	drinking	defecation frequency	volume of feces	consistency of feces	color of feces	abdominal discomfort	flatulence
	0 = more quiet or lethargic 1 = normal 2 = overly active or playful	0 = anorectic 1 = normal 2 = overly hungry	0 = less than normal 1 = normal 2 = more than normal	0 = zero 1 = one 2 = two 3 = three 4 = four 5 = five or more	0 = normal 1 =copious 2 = very copious	0 = hard 1 = normal 2 = pulpy 3 = loose, watery	0 = darker than normal 1 = normal 2 = gray 3 = yellowish	0 = no 1 = some 2 = frequent	0 = no 1 = some 2 = frequent

Figure 32: Daily patient owner log

Table 5: Veterinarian follow-up questionnaire results

Patients 12mg/kg	Body weight pre-treatment (kg)	Body weight post-treatment (kg)	Cobalamin supplementation Y/N	Quality of life
AH	10.4	NA	N	NA
CH	21.8	NA	Y	NC
CM	5.8	5.9	N	NC
CC	18.6	NA	N	NA
DE	6.3	6.6	N	I
DG	19.0	NA	N	I
GS	5.0	NA	Y	NA
LM	23.6	NA	Y	NC
LR	10.4	9.66	N	I
LS	5.0	7.8	N	NA
MD	9.8	10	N	NC
PF	9.1	NA	N	I

Patients 24mg/kg	Body weight pre-treatment (kg)	Body weight post-treatment (kg)	Cobalamin supplementation Y/N	Quality of life
BD	38.4	39	N	NC
BH	36.3	41.19	N	I
BP	16.4	14.6	Y	I
ES	5.3	NA	N	NC
GM	23.6	22.3	N	I
JW	8.5	8.4	N	NC
JW	19.3	NA	N	I
KA	8.5	8.72	N	I
LW	4.9	NA	N	I
LM	35.6	32	N	I
MG	2.9	2.92	N	I
MM	5.7	5.2	N	NC
MW	3.9	3.9	N	I
ND	5.2	5.6	N	NC
NA	4.7	5.13	N	I
PD	11.0	10.43	N	NC
PM	21.8	22.2	Y	I
SL	8.7	NA	N	I
ZS	20.4	19	N	I

Y=yes; N=no; NA=data not available; NC=no change; I=improvement

Table 8: Individual GI panel results 12 mg/kg

	cPLI µg/L		TLI µg/L		Cobalamin		Folate µg/L	
Patients 12 mg/kg	**Pre-treatment**	**Post-treatment**	**Pre-treatment**	**Post-treatment**	**Pre-treatment**	**Post-treatment**	**Pre-treatment**	**Post-treatment**
AH	381	696	21.3	39.1	843	725	21.5	27
CH	344	383	19.5	19.6	802	581	10.5	11
CM	6052	2836	44.2	54.8	855	583	12.5	8.9
CC	482	289	25.6	25.1	1000	322	11.1	14.6
DE	1000	426	32.5	23.5	742	741	9.3	16.1
DG	696	468	21.7	21.3	1000	354	72.1	26.8
GS	604	427	38.7	41.5	1000	887	20.8	10.6
LM	538	594	20.7	17.2	868	1001	16.9	15.1
LR	562	125	92	38.6	1001	1001	16.5	11.4
LS	493	1228	20.6	35.8	704	688	10.7	7.8
MD	509	902	40	27.6	829	852	72.1	59.9
PF	392	437	20.6	17.3	771	576	13	14.3

Table 9: Individual GI panel results 24mg/kg

	cPLI µg/L		TLI µg/L		Cobalamin		Folate µg/L	
Patients 24 mg/kg	**Pre-treatment**	**Post-treatment**	**Pre-treatment**	**Post-treatment**	**Pre-treatment**	**Post-treatment**	**Pre-treatment**	**Post-treatment**
BD	1374	1490	22.3	24.2	884	681	5.5	5.4
BH	1278	425	87.9	17.1	1000	1000	22.8	17.8
BP	459	514	96.5	18.4	632	308	9.9	15.2
ES	414	371	41.1	28.5	262	239	42.7	35.4
GM	965	703	31.9	40.3	620	438	17.1	16.4
JW	475	295	14.9	19.4	870	740	16.8	23.2
JW	847	734	22.4	33.7	449	1001	17	17.4
KA	1344	633	41	80.5	1000	804	21.9	26.4
LW	652	331	101	19.4	566	589	14	16.8
LM	918	637	34.7	5.7	762	311	20.7	16
MG	842	288	34.7	32.3	568	681	19.8	23.8
MM	992	370	15.9	13.7	1001	675	23.2	50.9
MW	742	193	16	19.7	912	857	12	16.8
ND	1960	2580	101	47.2	930	609	14.6	17.7
NA	475	520	16.6	101	953	802	22.3	19.9
PD	882	776	22.9	26.7	738	488	22.7	23.4
PM	717	325	101	14.1	385	220	22	19.1
SL	2024	822	62.6	29.1	1001	1001	23.8	46.8
ZS	690	996	18.6	25.5	706	725	14.7	14.4

Table 10: Individual CrP, S100A12 results 12 mg/kg

	CrP mg/L		S100A12 µg/L	
Patients 12 mg/kg	**Pre-treatment**	**Post-treatment**	**Pre-treatment**	**Post-treatment**
AH	9	3.9	214	87
CH	30.3	9.4	52	43
CM	23.8	5.1	380	213
CC	0.7	0.4	89	85
DE	0.4	0.1	144	88
DG	0.2	0.1	66	53
GS	1.2	0	404	246
LM	0.1	0.4	240	236
LR	1.2	1.5	1327	449
LS	0.1	0.1	160	72
MD	0.9	0.7	147	124
PF	0.1	2	x	x

Table 11: Individual CrP, S100A12 results 24 mg/kg

	CrP mg/L		S100A12 µg/L	
Patients 24 mg/kg	**Pre-treatment**	**Post-treatment**	**Pre-treatment**	**Post-treatment**
BD	0.1	0.1	78	82
BH	0.1	0.1	238	116
BP	1.6	0.1	99	380
ES	16	6.8	95	126
GM	3.6	0.1	135	137
JW	0.7	0.1	174	312
JW	0.1	0.1	41	58
KA	2.5	0.1	973	277
LW	0.1	0.9	121	241
LM	0.1	0.1	147	93
MG	11.2	0.1	384	436
MM	0.1	0.1	281	177
MW	0.1	0.1	67	95
ND	0.1	0.1	142	129
NA	0.1	6.3	232	148
PD	0.1	1.5	813	709
PM	0.1	0.1	291	746
SL	0.8	0.1	x	x
ZS	4.2	0.1	x	x

Table 12: Serum biochemistry tx camostat mesilate 12 mg/kg/d

	GLU mg/dL		BUN mg/dL		CREA mg/dL		CAL mg/dL		PHOS mg/dL		ALB g/dL		TPRO g/dL		TBILI mg/dL	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
AH	128	107	25	28	0.8	1	11.6	10.9	4.2	4.1	3.4	3.5	6.8	7	0.1	0.1
CH	84	86	13	14	0.8	0.8	9.6	10	3.7	3.6	3.1	2.9	5.3	5.3	0.1	0.2
CM	97	75	19	16	0.8	0.7	10.1	9.5	4	3.8	3.5	3.3	5.8	5.6	0.2	0.1
CC	104	102	11	15	1	0.6	9.6	9.1	3	2.9	2.7	2.7	5.1	5.1	0.1	0.1
DE	98	99	18	19	1.7	1.6	10	9.4	4.1	3.2	2.8	2.7	5.9	5.8	0.2	0.2
DG	78	78	11	12	0.8	1	10.2	10.5	4	4.4	3.1	3.1	6.4	6.4	0.1	0.2
GS	95	101	35	27	0.7	0.5	10.8	10.1	4.2	8.3	2.9	3.4	5.9	7.2	0.1	0
LM	54	74	11	9	0.6	0.5	9.9	9.5	4.3	4.7	3.2	3.2	5.9	6.2	0.1	0
LR	92	89	15	20	1	0.8	10.3	9.6	3.6	3.6	3	3	5.2	5.9	0.2	0.2
LS	81	86	14	19	0.7	0.7	8.3	8.6	3.1	5.4	2.4	2.2	3.7	3.8	0	0
MD	47	89	15	17	1.1	0.7	10.9	9.8	4.1	5.4	3.4	2.8	5.9	5.4	0.1	0.1
PF	73	87	13	14	0.8	0.6	9.9	10.5	3.6	9.4	3.1	3.7	6.2	7.7	0.1	0

	ALP U/L		ALT U/L		AST U/L		GGT U/L		CHOL mg/dL		TRIG mg/dL		GLOB g/dL	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
AH	2327	1538	114	101	18	22	5	7	296	246	219	100	3.3	5.5
CH	193	199	49	37	16	15	9	9	201	205	59	7	2.2	2.4
CM	17	18	27	26	37	24	3	6	191	178	74	48	2.3	2.4
CS	29	22	18	18	31	26	8	5	117	189	66	53	2.4	2.5
DE	171	218	60	72	23	22	7	6	259	233	98	88	3.1	3.1
DG	2186	2010	195	124	44	41	21	15	751	780	504	640	3.3	3.3
GS	189	247	52	143	24	18	6	5	172	231	146	1109	2.9	3.8
LM	337	561	372	514	52	47	24	64	297	458	161	210	2.7	3.1
PF	42	87	42	35	31	32	13	5	173	191	58	71	2.2	2.9
LR	35	221	28	70	26	73	4	7	123	154	86	80	1.3	1.5
LS	185	117	25	20	30	24	7	10	254	232	148	109	2.5	2.5
MD	80	194	30	21	26	8	7	5	220	390	201	1305	3.1	2.4

GLU= Glucose, BUN= Blood urea nitrogen, CAL= Calcium, PHOS= Phosphorus, ALB= Albumin, TPRO= Total protein, TBILI= Total bilirubin, ALP= Alkaline phosphatase, ALT= Alanine amino transferase, AST= Aspartate amino transferase, GGT= Gamma glutamyl transferase, CHOL= Cholesterol, TRIG= Triglycerides, GLOB= Globulin

Table 13: Serum biochemistry tx camostat mesilate 24 mg/kg/d

	GLU mg/dL		BUN mg/dL		CREA mg/dL		CAL mg/dL		PHOS mg/dL		ALB g/dL		TPRO g/dL		TBILI mg/dL	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
BD	89	83	17	17	1.4	0.9	8.3	9	3.4	4.2	2.3	2.6	3.7	4.3	0.2	0.1
BH	108	91	22	21	1.1	1.3	11.2	10.3	4.6	4	3.5	3.1	6.3	5.8	0.1	0.1
BP	107	118	47	24	1	1.3	10	9.6	3.8	4.3	3.1	2.7	5.7	4.9	0.2	0.1
ES	105	98	14	13	0.7	0.8	10.4	10.6	3.6	3.1	3.3	3.2	5.7	5.6	0.2	0.2
GM	105	95	13	12	1	1.1	8.6	9.2	4.7	5.1	2.5	2.3	4.5	5.1	0.1	0.1
JW	95	97	24	26.6	1.1	0.81	11.6	11.1	3.2	3.15	3.9	4.5	6.9	6.9	0.1	0.1
JW	108	95	13	12	0.8	0.5	9.7	10.1	3.9	4.3	3.2	3.4	5.6	5.8	0.2	0.2
KA	81	98	15	19	0.8	0.7	10.6	10.5	3.5	4.4	3.4	3.6	6	6	0.1	0
LW	83	90	13	14	1	1.1	9.7	9.4	3	3.5	3.5	3.3	5.8	5.2	0.2	0.2
LM	91	67	19	20	0.9	1	10.1	9.6	4.3	5.6	3.1	3.9	5.9	6.9	0.1	0.2
MG	91	83	16.7	27	0.7	1	7.4	10	4.3	4.1	2.5	2.4	4.9	4.3	0.1	0.1
MM	85	99	27.6	28	1.7	0	10.7	10.9	5	4.7	3.3	2.9	5.1	5.2	0.1	0.1
MW	97	84	14	9.7	0.7	0.8	10.5	10.8	3	3.8	3.5	4.6	6.2	7.2	0.2	0.1
ND	92	89	33	35	1.7	1.7	11.3	9.9	5.1	4.4	3.4	3	6.2	5.4	0.1	0
NA	83	x	34	21	1.4	1.1	11.4	10.2	3.6	2.9	3.2	2.1	5.9	4.7	0	0
PD	96	104	18	17	0.7	0.7	10.4	9.6	3.8	4.3	3.2	2.6	5.5	4.9	0.2	0.1
PM	95	85	15.6	18	0.93	1.1	10.1	10.1	4.5	5.2	3.6	3.2	5.8	5.7	0.1	0.2
SL	101	96	17	22	1.6	1.4	11.2	10.8	4.4	3.1	3.2	3	6.6	5.9	0.8	0.1
ZS	103	86	30	26	0.6	0.7	9.2	9.9	3.6	4.6	2.1	2.4	5	4.7	0	0

	ALP U/L		ALT U/L		AST U/L		GGT U/L		CHOL mg/dL		TRIG mg/dL		GLOB g/dL	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
BD	45	116	29	71	35	35	5	32	252	264	122	79	1.7	1.7
BH	373	324	141	86	27	24	12	12	283	206	203	144	2.6	2.9
BP	169	153	98	46	20	16	4	6	249	234	119	124	2.6	2.2
ES	160	71	252	80	26	20	23	17	176	119	65	74	2.4	2.4
GM	25	61	52	53	23	20	4	4	207	206	99	88	2	2.8
JW	339	369	79	50	38	23	28	6.2	196	196	122	112	3	2.4
JW	181	249	123	67	35	36	8	10	157	215	87	149	2.3	2.4
KA	193	134	77	74	23	22	9	7	210	211	99	323	2.6	2.4
LW	82	58	26	32	31	30	4	6	200	207	73	59	2.3	2
LM	242	212	207	50	36	178	5	177	222	210	112	100	2.8	3
MG	444	313	229	201	49	41	28	14	136	233	137	126	2.4	1.9
MM	218	279	52	62	17	24	3	6	170	192	143	75	1.8	2.3
MW	51	64	94	180	27	26	7	7	158	185	86	99	2.7	2.6
ND	701	400	183	114	33	35	4	7	280	270	151	313	2.8	2.4
NA	38	66	31	36	25	24	9	5	247	243	225	59	2.7	2.6
PD	125	217	149	107	28	18	7	6	299	314	117	141	2.3	2.2
PM	40	72	31	42	21	30	3	1	230	233	234	220	2.2	2.6
SL	401	128	174	55	6	20	19	6	246	265	413	109	3.4	2.9
ZS	45	139	25	38	20	27	1	5	347	370	102	225	2.6	2.3

GLU=Glucose, BUN=Blood urea nitrogen, CAL=Calcium, PHOS=Phosphorus, ALB=Albumin, TPRO=Total protein, TBILI=Total bilirubin, ALP=Alkaline phosphatase, ALT=Alanine amino transferase, AST=Aspartate amino transferase, GGT= Gamma glutamyl transferase, CHOL=Cholesterol, TRIG=Triglycerides, GLOB=Globulin

Table 14: Serum electrolytes tx camostat mesilate 12 mg/kg/d

	Sodium meq/L		Potassium meq/L		Na/K Ratio		Chloride meq/L	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
AH	148	146	5.8	5.6	25.5	26.1	106	110
CH	145	146	4.9	4.8	29.6	30.4	112	112
CM	148	148	5.2	5.2	28.5	28.5	109	113
CC	151	147	5.2	4.6	29	32	117	116
DE	145	145	4.8	4.6	30.2	31.5	115	115
DG	146	144	5.1	5.3	28.6	27.2	108	108
GS	148	143	4.8	5.3	30.8	27	116	114
LM	148	146	5.6	5.8	26.4	25.2	109	106
LR	145	146	4.6	4.8	31.5	30.4	109	107
LS	146	145	5	5.4	29.2	26.9	110	107
MD	141	146	4.3	5.3	32.8	27.5	106	115
PF	150	149	4.8	4.6	31.3	32.4	107	107

Table 15: Serum electrolytes tx camostat mesilate 24 mg/kg/d

	Sodium meq/L		Potassium meq/L		Na/K Ratio		Chloride meq/L	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
BD	148	147	5.3	5.2	27.9	28.3	115	115
BH	149	147	4.7	5	31.7	29.4	109	111
BP	151	148	4.3	4.8	35.1	30.8	119	118
ES	150	149	4.8	5.1	31.3	29.2	110	111
GM	145	150	4.6	4.9	31.5	30.6	113	114
JW	151	148	5.2	4.9	29	30.2	106	111
JW	146	147	4.9	5.4	29.8	27.2	107	108
KA	147	147	5.3	4.5	27.2	32.7	111	112
LW	149	147	4.4	4.9	33.9	30	112	113
LM	147	142	5.2	5.3	28.3	26.8	110	104
MG	148	149	5.7	5.4	26	27.6	112	109
MM	146	146	4.7	4.6	31.1	31.7	110	109
MW	147	147	4.6	5.2	32	28.3	110	109
ND	150	149	5.2	4.9	28.8	30.4	113	113
NA	146	147	4.8	5	30.4	29.4	110	114
PD	147	144	6	5.9	24.5	24.4	111	110
PM	148	148	4.5	4.9	32.9	30.2	113	114
SL	149	151	4.9	5.4	30.4	28	119	113
ZS	147	148	5	4.7	29.4	31.2	108	112

Table 16: Complete blood count tx camostat mesilate 12 mg/kg/d

	RBCx M/µL		HGB g/dL		HCT %		MCV fL		MCH pg		MCHC g/dL		PLT x K/µL	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
AH	5.4	5.43	12.6	12.1	39	36.8	71.8	68.1	22.4	23.2	32.3	32.9	620	483
CH	5.66	5.91	12.9	13.2	40	40.4	67.7	71.4	23.3	21.8	32.3	32.7	193	244
CM	9	9.29	21.7	21	63.2	61.5	68	68.3	23.3	23.4	34.3	34.1	438	340
CC	6.53	7.48	15.9	14	50.7	45.4	67.8	69.5	21.4	21.3	31.4	30.8	324	252
DE	7.89	5.89	17.9	13.5	57.3	43	72.6	73	22.7	22.9	31.2	31.4	227	462
DG	7.08	6.8	15.6	15.6	49.9	48.3	70.5	71	22	22.9	31.3	32.3	263	202
GS	5.61	5.63	13.4	14.3	42.9	45.2	76.5	80.3	23.9	25.4	31.2	31.6	250	451
LM	6.54	6.06	15.1	14.2	46	44.4	70.3	73.3	23.1	23.4	32.8	32	339	418
LR	6.87	7.24	17.2	17.6	50.3	54	73.2	74.6	25	24.3	34.2	32.6	429	255
LS	4.97	3.29	10.4	8.55	34.1	24.4	68.6	74.2	20.9	26	30.5	35	501	549
MD	6.58	6.59	16.5	16.2	51.6	50	78.4	75.9	25.1	24.6	32	32.4	187	203
PF	7.12	7.03	16.2	17.6	50	50.4	70.2	71.7	22.8	25	32.4	34.9	240	262

	Total WBC		Netrophils %		Lymphocytes %		Monocytes %		Eosinophils %		Neutrophils /µL		Lymphocytes /µL		Monocytes /µL		Eosinophils /µL	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
AH	8160	3420	81	77	14	19	2	1	3	3	6610	2633	650	1142	163	34	245	103
CH	7700	7050	69	65	26	19	1	7	4	8	5313	4583	1340	2002	77	494	308	564
CM	5500	5110	74	54	19	29	4	6	3	11	4107	2759	1482	1055	222	307	167	562
CS	8080	5150	63	72	22	18	8	5	7	5	5090	3708	927	1778	646	258	566	258
DE	6400	6160	64	67	18	13	11	10	7	10	4096	4127	1152	801	704	616	448	616
DG	4850	4310	72	77	16	15	6	3	6	5	3492	3319	776	647	291	129	291	216
GS	11500	12700	57	77	31	20	3	1	9	2	6555	9779	3565	2540	345	127	1035	254
LM	10200	7600	89	73	5	6	5	16	1	0	9078	5548	510	456	510	1216	102	0
PF	13200	11100	83	84	11	9	3	5	2	2	10956	9324	1452	999	396	555	264	222
LR	10100	12500	68	72	21	10	7	4	4	13	6868	9000	2121	1250	707	500	404	1625
LS	10200	13600	60	84	22	8	7	1	11	7	6120	11424	2244	1088	714	136	1122	952
MD	5400	8190	47	50	39	34	2	3	12	13	2538	4095	2106	2785	108	246	648	1065

RBC = Red blood cells, HGB = Hemoglobin. HCT = Hematocrit, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemogloin, MCHC = mean corpuscular hemoglobin concentration, PLT = Platelet estimate

Table 17: Complete blood count 1/2 tx camostat mesilate 24 mg/kg/d

	RBCx M/µL		HGB g/dL		HCT %		MCV fL		MCH pg		MCHC g/dL		PLT x K/µL	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
BD	6.04	5.81	14	14.2	45.9	43.2	76	74.4	23.5	24.1	30.9	32.4	440	238
BH	5.36	5.84	14.8	14.1	44.2	51.2	82.5	87.8	26.3	25.3	31.9	28.9	689	407
BP	8.02	7.96	18.8	18.2	54.9	53.5	68.5	67.2	22.7	23.6	33.2	35.1	263	291
ES	7.8	8.24	19.5	19.4	58.8	60.3	75.4	73.2	24.9	23.7	33	32.3	470	486
GM	6.42	6.49	15.3	15.5	45.3	48.8	70.6	75.2	22.8	23.9	33.8	31.8	295	399
JW	7.14	7.56	17.1	18.4	55	55.1	77	72.9	23.9	24.3	31.1	33.4	324	325
JW	6.05	6.07	11.1	13	35.9	42.2	59.3	69.5	18.3	21.4	30.9	30.8	591	482
KA	6.66	7.81	16.4	19	53	59	79.6	75.5	24.6	24.3	30.9	32.2	285	357
LW	8.81	9.07	20.3	21	64.6	67	73.3	73.9	23	23.2	31.4	31.3	376	328
LM	6.01	5.96	15.5	14.6	44.9	45.1	73.7	75.7	25.5	24.5	34.5	32.4	450	319
MG		5.99		20.1		48		69.7		24.4		31.1		341
MM	7.87	7.94	17.4	18.2	55	54.3	69.9	68.4	22.1	22.9	31.6	33.5	434	574
MW	7.98	8.77	19.6	21.3	59.2	62.8	74.2	71.6	24.6	24.3	33.1	33.9	177	299
ND	8.1	7.07	16.4	14.6	50.4	46.9	62.3	66.3	20.3	20.7	32.5	31.1	417	454
NA	6.85	7.22	16.2	17.9	51.8	54.2	75.6	75.1	23.6	24.8	31.3	33	389	247
PD	5.73	5.82	13.5	14	46.9	44	81.8	75.6	23.6	24.1	28.8	31.8	273	234
PM	6.74	7.24	16	16.7	50	51.9	74.2	71.7	23.7	23.1	32	32.2	562	315
SL	7.26	6.88	17	16.5	53	50	73	72.7	23.4	24	32.1	33	273	217
ZS	6.95	7.07	18.3	18.6	56.8	58	81.7	82	26.3	26.3	32.2	32.1	264	218

RBC = Red blood cells, HGB = Hemoglobin. HCT = Hematocrit, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemogloin, MCHC = mean corpuscular hemoglobin concentration, PLT = Platelet estimate

Table 18: Complete blood count 2/2 tx camostat mesilate 24 mg/kg/d

	Total WBC		Netrophils %		Lymphocytes %		Monocytes %		Eosinophils %		Neutrophils /µL		Lymphocytes /µL		Monocytes /µL		Eosinophils /µL	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
BD	12800	7580	84	84	7	6	7	6	2	4	10752	6367	896	455	896	455	256	303
BH	14000	6290	84	78	3	7	12	1	1	14	11760	4906	420	440	1680	63	140	881
BP	9480	6910	80	74	5	9	7	8	8	9	7584	5113	474	622	664	553	758	622
ES	16800	8160	87	75	7	16	6	6	0	3	14616	6120	1176	1306	1008	490	0	245
GM	7040	8450	75	79	18	18	6	3	0	0	5280	6676	1267	1521	422	254	0	0
JW	6630	9010	81	72	9	9	8	10	2	6	5370	6487	597	811	530	901	133	541
JW	6820	6640	93	86	5	7	2	5	0	2	6343	5710	341	465	136	332	0	133
KA	4150	3920	75	54	23	25	x	14	2	7	3113	2117	955	980	0	549	83	274
LW	3740	4530	75	61	15	31	7	3	3	5	2805	2763	561	1404	262	136	112	227
LM	8100	11100	72	83	14	3	7	7	7	7	5832	9213	1134	333	567	777	567	777
MG	11500		65		30		1		4		7475		3450		115		460	
MM	5100	5850	75	86	13	8	8	4	4	2	3825	5031	663	468	408	234	204	117
MW	5650	6250	71	71	17	23	5	5	7	1	4012	4438	961	1438	283	313	396	63
ND	7240	7750	60	74	29	17	3	2	8	7	4344	5735	2100	1318	217	155	579	543
NA	7500	5710	65	76	20	10	11	9	4	5	4875	4339	1500	571	825	514	300	286
PD	8120	9960	72	75	7	8	10	8	10	9	5846	7470	568	797	812	797	812	896
PM	8050	10300	72	69	20	18	2	7	6	6	5796	7107	1610	1854	161	721	483	618
SL	7250	7100	51	56	42	40	6	4	1	0	3698	3976	3045	2840	435	284	73	0
ZS	5740	6000	73	70	15	18	7	5	5	7	4190	4200	861	1080	402	300	287	420

12 PUBLICATIONS

This work is unpublished.

13 DANKSAGUNG

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14 SELBSTÄNDIGKEITSERKLÄRUNG

Hiermit bestätige ich die vorliegende Arbeit selbstständig angefertigt zu haben. Ich versichere ausschliesslich die angegebenen Quellen und Hilfen in Anspruch genommen zu haben.

Berlin, den 28.05.2015 Tim Kretzschmar