COMPARISON OF EFFECTS OF DAILY AND PULSE DOSE CALCITRIOL IN TREATING RENAL SECONDARY HYPERPARATHYROIDISM IN CATS WITH PRIMARY CHRONIC RENAL FAILURE

A Thesis
Presented in Partial Fulfillment of the Requirements for The Degree Master of Science in the Graduate School of The Ohio State University

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ABSTRACT

The primary objective of this study was to determine the efficacy of calcitriol in controlling renal secondary hyperparathyroidism in cats with naturally-occurring chronic renal failure (CRF). Other objectives included comparing the effectiveness of daily versus pulse dose (every 84 hours) calcitriol in controlling renal secondary hyperparathyroidism and evaluating for adverse effects of calcitriol administration, primarily hypercalcemia.

Two groups of cats were studied. The groups consisted of 10 normal cats and 10 cats with CRF. Normal cats were defined as those having no azotemia (abnormally high blood urea nitrogen or serum creatinine concentrations), negative urine culture on urine obtained by cystocentesis, and normal physical examination. Cats with CRF were included if abnormally high serum creatinine concentration, >1.8 mg/dL and <5.0 mg/dL, and isosthenuria were present, and if serum phosphorus concentration was <6.0 mg/dL.

Both groups of cats were treated in an identical manner. Before beginning the study, baseline data including packed cell volume, total protein concentration, serum biochemistry, urinalysis, urine culture on urine obtained by cystocentesis, serum ionized calcium concentration, serum parathyroid hormone (PTH) concentration, and serum calcitriol concentration were obtained. In the first phase of the study, each cat was given calcitriol at a dosage of 2.5 ng/kg daily for 14 days. After a 14 day washout
period, the second phase of the study was started and each cat was given calcitriol at a dosage of 8.75 ng/kg every 84 hours (h). On days 1, 2, and 3 after starting calcitriol during each phase, serum ionized calcium concentration was determined to identify development of hypercalcemia. On day 14 of each phase, serum concentrations of ionized calcium, calcitriol, and PTH were determined before calcitriol administration (time 0), and 2, 4, and 6h after calcitriol administration.

PTH concentrations were significantly higher in the CRF group when compared to the normal group of cats at the beginning of the study. Serum PTH concentrations decreased in both groups of cats during both phases of the study. At the end of phase 1, PTH concentration had decreased significantly in the CRF cats when compared to values obtained before enrollment in the study. In the CRF group, serum PTH concentration at the end of phase 2 was identical to the value obtained at the end of phase 1. Calcitriol administration appeared to help alleviate renal secondary hyperparathyroidism regardless of the dosage regimen used. Further study is warranted to determine optimal dosage and to evaluate the use of pulse dosing in CRF cats with a maximally hyperplastic parathyroid glands. Hypercalcemia, a potential complication of calcitriol administration, was not observed in any cat throughout the study period with either dosage protocol.
Dedicated to my parents,

for their encouragement and understanding through the years and through my professional training.
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CHAPTER 1
LITERATURE REVIEW AND INTRODUCTION

1.1 Literature Review

1.1.1 Chronic Renal Failure

Chronic renal failure (CRF) is one of the most common diseases of older cats. It is progressive in nature, irreversible, characterized by abnormal renal architecture, and generally leads to death of the affected cat. The age of onset of CRF has been evaluated in many studies. One study found the mean age of onset in 80 cats to be 12.6 years.\(^1\) Another study found 37% were younger than 10 years, 31% were between 10 and 15 years, and 32% were greater than 15 years of age.\(^2\) Although there is no known cure, affected cats may survive for a prolonged period of time through supportive care, biochemical monitoring, and client education.

Several different disease processes within the kidney may cause CRF. Familial renal diseases such as amyloidosis in Abyssinian,\(^3\) Siamese, and Oriental Shorthair cats and polycystic kidney disease in Persian cats\(^4\) can cause CRF. Congenital renal disease present at birth may result from genetic abnormalities or exposure to harmful factors in utero. The acquired form of CRF is most common. The underlying cause in most cases is unknown and histological lesions typically are those of chronic tubulointerstitial nephritis including lymphoplasmacytic inflammation and interstitial nephritis. One
study of 47 cats showed tubulointerstitial nephritis to be the most common cause of acquired CRF, affecting 70.4% of cats. Any disease process, such as infection or toxin exposure, that causes irreversible damage to renal parenchyma may cause acquired renal failure. If the animal survives the acute renal insult, CRF may result if sufficient renal mass was irreversibly damaged to curtail adequate function.

1.1.1.2 Chronic Renal Failure Management

The goal of patient management in CRF is to improve the quality of life and promote longevity hopefully by slowing progression of the underlying renal disease. This can be accomplished in part by controlling the clinical consequences of the disease and monitoring biochemical parameters that may accompany disease progression. Clinical signs of CRF include anorexia, weight loss, vomiting, polyuria, polydipsia, depression, blindness due to retinal hemorrhage or detachment associated with hypertension, and cervical ventroflexion due to hypokalemia. Laboratory abnormalities that may contribute to clinical signs or lead to progression of disease include anemia, hyperphosphatemia, metabolic acidosis, hypokalemia, hypertension and renal secondary hyperparathyroidism.

Many of the clinical signs of renal failure are considered to be secondary to the accumulation of uremic toxins, or excess loss of other substances into the urine. Blood urea nitrogen and serum creatinine concentrations are the classically measured parameters of uremia, but other substances also may accumulate or become deficient and lead to clinical signs. Parathyroid hormone (PTH) has been well documented as a uremic toxin.
Anorexia and vomiting seen with CRF are multifactorial in origin. CRF leads to decreased clearance or degradation of many substances such as gastrin, guanidine, and urea. The decreased degradation of gastrin by the kidneys leads to increased serum gastrin concentrations and secondary gastric hyperacidity with associated anorexia and vomiting. Decreased guanidine clearance stimulates the chemoreceptor trigger zone and also contributes to anorexia and vomiting. Other complicating factors include dehydration, metabolic acidosis, anemia, and hypokalemia which can cause weakness and inappetance. Increased serum urea concentration allows this substance to be degraded by normal bacterial flora, especially in the mouth. After degradation of urea by normal flora, ammonia is liberated and painful oral mucosal ulceration follows.

Polyuria primarily is caused by a decreased ability of the kidney to adequately concentrate urine and impaired ability to respond to antidiuretic hormone. As a consequence of this decreased urine concentrating ability, the affected cat must consume more water to maintain adequate hydration (i.e. polydipsia). If adequate compensatory polydipsia does not occur, dehydration may ensue. Dehydration further exacerbates the magnitude of azotemia, and clinical signs may worsen.

Medical management of clinical signs may include the use of antiemetics for vomiting, and anorexia; the use of parenteral fluids to maintain hydration; placement of gastrotomy or esophagostomy tubes to provide nutrition, fluids, and medications; the use of human recombinant erythropoietin for anemia; and the provision of alkali and potassium to combat acidosis and hypokalemia, respectively. Medications such as potassium citrate provide a source of potassium and alkali, and may be used to combat both conditions. The severity of clinical signs may vary greatly among animals with
similar magnitudes of azotemia, and medical management should be individually tailored.

Laboratory analysis of cats with CRF should always include evaluation of serum potassium, bicarbonate, calcium, and phosphorus concentrations. Hypokalemia and acidosis associated with CRF may lead to weakness, vomiting, and progression of the disease. Disorders of serum calcium concentration commonly include a mild increase in serum total calcium concentration. The serum ionized calcium concentration generally is within normal limits, and in dogs the increase often is secondary to calcium complexed with anions that accumulate as renal failure develops. As CRF progresses to end stage, decreased serum ionized calcium concentration may ensue leading to clinical signs such as weakness and twitching.

CRF is the most common cause of hyperphosphatemia in cats and it is a common laboratory finding. Hyperphosphatemia generally is not seen early in the disease process due to a PTH mediated compensatory decrease in phosphorus reabsorption by the remaining tubules. When approximately 80-85% of the nephrons are no longer functional, increased serum phosphorus concentration occurs. The consequences of hyperphosphatemia can be detrimental. Hyperphosphatemia may lead to hypocalcemia, soft tissue mineralization (especially when the calcium X phosphorus product exceeds 60-70), and aids in the development of renal secondary hyperparathyroidism. Controlling serum phosphorus concentration is paramount in slowing the progression of CRF. Hyperphosphatemia may be treated, or prevented, by decreasing phosphorus intake in the diet, orally administering phosphorus binders, and maintaining adequate hydration. In addition to hypocalcemia and soft tissue
mineralization, hyperphosphatemia causes disturbances in calcium homeostasis including alteration of concentrations of calcitriol and parathyroid hormone (PTH), and therefore the adverse effects of renal secondary hyperparathyroidism.¹⁴

1.1.2 Calcium Homeostasis

The total serum calcium concentration, which is routinely measured in the laboratory, is comprised of 3 fractions. The largest is the non-functional protein-bound fraction, which generally comprises 35% of the total calcium and serves as a reservoir for ionized calcium. The ionized calcium fraction is the functional, actively regulated portion, and comprises approximately 55% of the total calcium. The remaining fraction includes calcium that is complexed with small molecular weight anions. This portion comprises approximately 10% of the total calcium.¹¹

The ionized calcium concentration is the fraction that is closely regulated by the body as it is the fraction with biological activity.¹³,¹⁵ Regulation to maintain a narrow concentration range of this ion involves the interactions of calcitriol, PTH, calcitonin, and ionized calcium itself. As serum concentrations of ionized calcium decrease, potentially as a result of hyperphosphatemia, this change is sensed by the parathyroid gland and PTH is subsequently secreted and synthesis increased.¹⁶ The calcium receptor on the parathyroid gland senses increased extracellular ionized calcium concentration and elevates intracellular ionized calcium concentration.¹⁵ Increased intracellular calcium will directly inhibit the transcription of PTH DNA to mRNA within the cell nucleus of the parathyroid glands.¹⁷ The converse occurs in the face of ionized hypocalcemia. Calcitriol is important in the regulation of PTH secretion. Calcitriol will inhibit PTH secretion by elevating ionized calcium in blood. It is also
responsible as a regulator of gene expression for maintaining the number of calcium receptors on the secretory cells of the parathyroid gland.\textsuperscript{18,19} However, in the face of hyperphosphatemia, with adequate calcitriol, PTH suppression cannot occur due in part to post-transcriptional stabilization and decreased degradation of synthesized mRNA for the PTH hormone.\textsuperscript{20} Normocalcemia and the presence of calcitriol are both essential for negative feedback on the transcription of the PTH gene within the nucleus.\textsuperscript{18} A portion of the effect of hyperphosphatemia is due to its lowering of serum ionized calcium concentration which, via the calcium receptor, results in lowered cytosolic and nuclear calcium concentrations.\textsuperscript{18} Calcium has direct inhibitory effects on PTH secretion and stimulatory effects on intracellular degradation of active PTH\textsuperscript{15,20} as well as the mRNA coding for PTH.\textsuperscript{21}

PTH is secreted as an 84 amino acid polypeptide.\textsuperscript{22} The functional part of the hormone is the amino terminus that binds with PTH receptors throughout the body. The carboxy terminus is believed to solely aid in the secretory process.\textsuperscript{23} The goal of PTH secretion is to elevate extracellular ionized calcium concentration. PTH achieves this goal by direct action on the kidney and bone, and by indirect action in the intestine. PTH acts on the kidney by direct actions on the distal convoluted tubule where it stimulates calcium reabsorption,\textsuperscript{24} and through proximal tubular activation of the 1\textalpha-hydroxylase, which facilitates conversion of 25-hydroxyvitamin \textit{D}_{3} (calcidiol) to 1,25-hidroxyvitamin \textit{D}_{3} (calcitriol). Calcitriol so formed has direct effects on the intestine to stimulate calcium absorption. The immediate direct effects of PTH on bone include activation of existing bone cells to increase flow of calcium from bone to the circulation.\textsuperscript{25}
The main source of vitamin D in the cat is the diet because cats have limited ability to photosynthesize vitamin D in the skin. After absorption, vitamin D is converted to calcidiol in the liver and subsequently to calcitriol, the most active form, in the kidney. Calcitriol synthesis is stimulated by deficiencies of calcium, phosphorus, and calcitriol, and its synthesis is inhibited by excess calcium, calcitriol, and by phosphate loading. Calcitriol is needed by both the kidney and intestine to elevate serum calcium concentration, ensure bone mineralization, and prevent osteomalacia in adults.

Systemically, calcitriol exerts its primary action on the intestine where it stimulates synthesis of calbindin and enterocyte membrane pumps that result in calcium absorption. An important therapeutic concern is the potential ability of calcitriol to induce transcaltachia, which is an increase in serum ionized calcium due to rapid gastrointestinal absorption after oral calcitriol administration. In the bone, calcitriol is responsible for differentiation of hematopoietic precursors into osteoclasts, the primary bone-resorbing cell. Calcitriol stimulates bone resorption by stimulating osteoblasts to secrete substances that activate osteoclasts. In the kidney, calcitriol’s primary action is direct inhibition of 1α-hydroxylase and prevention of calcitriol overproduction. Calcitriol also aids in the tubular reabsorption of calcium and phosphorus from the glomerular filtrate.

Calcitriol has both direct and indirect effects on the parathyroid glands in regards to production of PTH. Directly, calcitriol will cause up regulation of vitamin D receptors, inhibit transcription of PTH by binding to the vitamin D receptor with simultaneous binding of ionized calcium to its receptor, and result in decreased PTH secretion. Indirectly, calcitriol will stimulate calcium absorption in the intestine or reabsorption
in the kidney, thus normalizing calcium and decreasing the stimulus for PTH secretion.

Calcitonin is the final hormone involved in calcium homeostasis. The primary function of calcitonin is on the bone where it inhibits osteoclastic activity during hypercalcemia. The role of calcitonin in daily calcium homeostasis is thought to be minimal, except after a calcium-rich meal.

1.1.3 Renal Secondary Hyperparathyroidism

The consistent development of renal secondary hyperparathyroidism in cats with CRF is multifactorial and complex in nature. As renal failure progresses, and hyperphosphatemia follows, the serum ionized calcium concentration subsequently is decreased. In addition to the development of hyperphosphatemia with progression of renal disease, there is an increasing loss of the ability of the kidney to synthesize calcitriol due to loss of the 1α-hydroxylase enzymes in the proximal convoluted tubule. Increased phosphorus, decreased calcium, and lack of calcitriol inhibition allow the parathyroid gland to secrete increasing amounts of PTH in order to maintain calcium homeostasis. Thus, as renal failure progresses, the parathyroid gland continues to become hyperplastic and secrete excessive amounts of PTH. The increase in PTH stimulates the remaining tubules to synthesize calcitriol, often returning the concentration of calcitriol to normal, but this response appears to be inadequate in suppressing PTH secretion. As the disease progresses to end stage, an absolute calcitriol deficiency develops due to a dramatically decreased functional renal mass.

Barber and Elliot demonstrated that in cats with compensated, uremic, and end stage CRF, the incidence of low serum calcitriol concentrations was 0%, 20%, and 80%
respectively, and the incidence on increased PTH concentrations was 45%, 86%, and 100% respectively.\textsuperscript{37} This study suggests that although serum calcitriol concentration may be normalized, there may be a relative deficiency when compared to the magnitude of PTH increase. Stated according to the ‘calcitriol trade-off hypothesis’,\textsuperscript{38,39} calcitriol is normalized only at the expense of an elevation of PTH.\textsuperscript{18}

The presence of an adequate serum calcitriol concentration is imperative in suppressing PTH secretion. If calcitriol is lacking and hyperplasia of the parathyroid glands has occurred, down regulation of both calcium and calcitriol receptors ensues\textsuperscript{40,41} and suppression of PTH secretion cannot occur even in the face of increased extracellular ionized calcium. In order to suppress transcription of the PTH gene and synthesis of PTH, both the calcium and\textsuperscript{17} vitamin D receptors in the nucleus must be occupied simultaneously.\textsuperscript{14,18} Therefore, with a relative or absolute lack of calcitriol, PTH concentration in blood cannot be suppressed.

\textbf{1.1.3.1 Treatment}

The negative effects of renal secondary hyperparathyroidism are multiple and its treatment should begin early in the course of renal disease. Renal secondary hyperparathyroidism can cause bone demineralization and soft tissue mineralization,\textsuperscript{14} and PTH can contribute to anemia\textsuperscript{42,43} and serve as a uremic toxin.\textsuperscript{6,7,18} Treatment of renal secondary hyperparathyroidism is a topic of intense research interest in both human and veterinary medicine. Previous treatments included subtotal parathyroidectomy, but current therapy includes the use of dietary phosphorus restriction, orally administered phosphorus binders, calcitriol, and other vitamin D metabolites.
Dietary phosphorus restriction appears to be an important adjunct in controlling renal secondary hyperparathyroidism. Elliot demonstrated its effectiveness by evaluating PTH and phosphorus concentrations in CRF with cats that were fed a normal diet and in those that were fed a diet formulated for treatment of CRF. Cats fed the phosphorus-restricted diet had an approximately 50% decrease in PTH and a 25% decrease in phosphorus concentration at the mid-survival point. The CRF cats that were not fed the phosphorus-restricted diet, had an approximately 80% increase in PTH and a 20% increase in phosphorus concentration at the mid-survival point. As renal failure progresses, dietary phosphorus restriction may become inadequate to prevent hyperphosphatemia and renal secondary hyperparathyroidism from developing.

Orally administered phosphorus binders are commonly used in veterinary medicine to prevent or treat hyperphosphatemia in cats with CRF. The most common oral phosphorus binder used in veterinary medicine is aluminum hydroxide. Aluminum-containing phosphorus binders are no longer used in human medicine due to the risk of aluminum toxicity, which may cause osteomalacia, anemia, and encephalopathy. Aluminum is minimally absorbed in the gastrointestinal tract, but absorption is increased with increased alkali content of the lumen of the intestine. Generally, the kidneys excrete excess aluminum, but impaired renal function in these patients after long-term use, which is common in hemodialyzed human patients, leads to aluminum toxicosis. Other available phosphorus binders include calcium-containing salts such as calcium citrate, calcium carbonate, and calcium acetate. However, in humans, calcium citrate is rarely used as a phosphorus binder due to its tendency to cause hypercalcemia and increase aluminum absorption due to the alkali...
component.\textsuperscript{51} Caution also must be exercised with its use in veterinary medicine due to the potential development of hypercalcemia. Sevelamer hydrochloride is a recently developed oral phosphorus binder that binds phosphorus by ion exchange and hydrogen bonding.\textsuperscript{46}

Currently, the treatment of renal secondary hyperparathyroidism in humans commonly includes the use of calcitriol or other vitamin D metabolites. The use of calcitriol also has been evaluated in veterinary medicine, but its use is not as common. Based on literature in human medicine, calcitriol and other vitamin D metabolites effectively control renal secondary hyperparathyroidism. One report in veterinary medicine found that approximately 85\% of calcitriol-treated cats had improved behavior and interactions with owners, 84\% had improvement of their appetite, 79\% were more active, and 88\% were perceived by their veterinarian to have longer life spans when compared to cats with CRF that were not treated with calcitriol.\textsuperscript{18}

Calcitriol aids in alleviating renal secondary hyperparathyroidism by several mechanisms. First, in order to adequately control renal secondary hyperparathyroidism with calcitriol, the serum phosphorus concentration must be normalized. Increased serum phosphorus concentration inhibits action of the 1\textalpha\-hydroxylase system, promotes soft tissue mineralization, and stimulates PTH release by decreasing serum ionized calcium concentration.\textsuperscript{36} If the serum phosphorus concentration is normalized and calcitriol is present, simultaneous binding of calcitriol and calcium to their respective receptors in the parathyroid cell nucleus suppresses transcription and synthesis of PTH. High serum phosphorus, among other effects, may complex ionic calcium disallowing this synergism with calcitriol. The end result of calcitriol administration is reversal or
prevention of parathyroid gland hyperplasia, and secondary normalization of PTH in uremic patients. Calcitriol also causes increased synthesis of calcium receptors on the chief cells of the parathyroid gland and upregulates systemic vitamin D receptors in uremic patients. These mechanisms participate in the effects of calcitriol to treat or prevent renal secondary hyperparathyroidism.

Calcitriol use has been evaluated using both daily and pulse dose therapy in humans with CRF and renal secondary hyperparathyroidism. Pulse dose therapy has been evaluated due to the potential to decrease the incidence of hypercalcemia. This expectation is due to the ability of gastrointestinal cells to respond to calcitriol stimulation only in newly formed cells as they leave the crypts of Lieberkühn. Cells within the crypt are replaced approximately every 24 hours and take approximately 4-6 days to reach the villus tips for extrusion. Therefore, when calcitriol is given at larger dosages but less frequently, there are fewer cells lining villi that are able to actively absorb calcium and the potential for hypercalcemia is decreased.

Studies in humans have demonstrated that calcitriol pulse therapy is just as effective as daily therapy in controlling renal secondary hyperparathyroidism in patients with CRF. In one study of 59 children with CRF, the mean PTH concentration decreased from 485 pg/ml to 232 pg/ml in the daily dose group and 315 pg/ml to 218 pg/ml in the pulse (twice per week) group. The mean decrease in PTH concentration was 19.2% and 13.7% respectively. There was no statistical difference between the groups at the end of the study. This approach resulted in resolution of hyperparathyroidism in 23/29 patients receiving daily therapy and 21/30 patients receiving pulse therapy. Another study
showed similar results and demonstrated that the use of calcitriol did not adversely affect growth in children.\textsuperscript{54}

1.2 Introduction

Although renal secondary hyperparathyroidism is a common occurrence in veterinary medicine, the use of calcitriol for its control in cats with naturally-occurring CRF has not been evaluated in veterinary medicine. Studies in humans have shown calcitriol's efficacy in controlling renal secondary hyperparathyroidism using both daily and pulse dose calcitriol. The use of calcitriol in human medicine is invariably done in combination with dietary phosphorus restriction alone or with orally administered phosphorus binders. The use of oral phosphorus restriction and phosphorus binders has been shown to be efficacious in controlling hyperphosphatemia and renal secondary hyperparathyroidism in veterinary medicine. However, the use of calcitriol was not studied in these reports. The potential to induce hypercalcemia with calcitriol administration may have prevented its routine use in veterinary medicine. The primary objectives of the following study were to evaluate the efficacy of calcitriol to control renal secondary hyperparathyroidism in cats with CRF, to evaluate the efficacy of daily and pulse dose therapy, to determine if hypercalcemia developed at the given dosages, and to evaluate the short-term effects of calcitriol on the concentrations of PTH, ionized calcium, and calcitriol itself.
CHAPTER 2

COMPARISON OF EFFECTS OF DAILY AND PULSE DOSE CALCITRIOL IN TREATING RENAL SECONDARY HYPERPARATHYROIDISM IN CATS WITH PRIMARY CHRONIC RENAL FAILURE

2.1 Introduction

In recent years, the number of cats evaluated for and diagnosed with primary chronic renal failure (CRF) has increased markedly. Given that there is no known cure, the primary goals of treatment include improving quality of life by controlling complications and correcting abnormalities, such as hyperphosphatemia and renal secondary hyperparathyroidism that may hasten disease progression. Complications that decrease quality of life include anorexia, lethargy, vomiting, anemia, and dehydration. Hyperphosphatemia and renal secondary hyperparathyroidism develop as CRF progresses, and their control is paramount in promoting longevity.

Renal failure is the most common cause of hyperphosphatemia in the cat\textsuperscript{12,13} and develops due to decreased phosphorus excretion by the tubules of the diseased kidneys. Early in renal disease, hyperphosphatemia may not be present due to increased phosphorus excretion by remnant nephrons secondary to increasing PTH as renal mass declines. As the percentage of functional nephrons decreases to less than 15-20% and dietary phosphorus intake is constant, hyperphosphatemia predictably develops.
Consequences of persistent hyperphosphatemia include hypocalcemia, soft tissue mineralization if the calcium X phosphorus product exceeds 60 to 70, and development of renal secondary hyperparathyroidism. Soft tissue mineralization (especially in the kidneys) then may lead to progression of the primary disease process. Hyperphosphatemia may be controlled by dietary phosphorus restriction, adequate hydration, and orally-administered phosphorus binders.

The development of renal secondary hyperparathyroidism in renal failure is multifactorial and the negative effects can be detrimental. Its development is caused by inhibition or lack of calcitriol production due to inhibition of 1α-hydroxylase by hyperphosphatemia or decreased functional renal mass, stimulation of PTH production due to decreased serum ionized calcium and calcitriol concentrations, and decreased number and responsiveness of vitamin D and calcium receptors in the parathyroid gland. Negative consequences of increased serum PTH concentration include bone demineralization, bone marrow suppression, encephalopathy, and anemia. Renal secondary hyperparathyroidism may be controlled by decreasing serum phosphorus concentration, administering calcitriol, or a combination of the two.

Evaluation of pulse dose therapy was incorporated into the study to determine its effectiveness in controlling renal secondary hyperparathyroidism as compared to daily dosing and to evaluate it effectiveness in preventing hypercalcemia. Calcitriol programming of enterocytes occurs only on newly formed cells leaving the crypt. Mature cells that were not exposed to calcitriol therefore will lack the ability to respond to calcitriol. Therefore, pulse dose therapy with calcitriol has been shown to decrease the occurrence of hypercalcemia in humans.
The purpose of this study was to evaluate the efficacy of calcitriol using either an oral dosage of 2.5 ng/kg every 24 hours (h), or a pulse dosage of 8.75 ng/kg every 84 h. Published dosages for calcitriol administration for renal secondary hyperparathyroidism range from 1.5 to 3.5 ng/kg in clinical dogs and cats. A midrange dosage of 2.5 ng/kg/day was chosen as it was believed to be efficacious in lowering elevated PTH while preventing hypercalcemia. A secondary objective was to evaluate pulse dosing to prevent adverse effects of calcitriol administration, primarily hypercalcemia. Ten cats with CRF and 10 normal control cats were enrolled in the study. We hypothesized that both dosages of calcitriol would adequately correct renal secondary hyperparathyroidism and that the incidence of hypercalcemia would be minimal or transient, particularly in pulse dosed circumstances.

2.2 Materials and Methods

2.2.1 Cats

Two groups of cats were enrolled in the study. Ten cats were determined to be normal based on history, physical examination, urinalysis and culture on urine obtained by cystocentesis, packed cell volume, total protein, and serum biochemistry profile. The sex of the normal cats enrolled included 6 castrated males, 3 intact females, and 1 spayed female. The age of the normal cats ranged from 3.6 to 12.2 years (mean, 7.3 years). The weight of the normal cats ranged from 3.2 to 7.8 kg (mean, 5.2 kg). Seven cats were client-owned and 3 cats were normal cats obtained from a research colony.

Ten cats diagnosed with CRF also were enrolled in the study. These cats were diagnosed with CRF based on the presence of inadequately concentrated urine (USG <1.020) in the presence of azotemia, abnormal renal size and architecture based on
abdominal ultrasonography, abdominal radiographs, and physical examination, and history. Cats with CRF were only enrolled if they were expected to live throughout the duration of the study, had a serum phosphorus concentration <6.0 mg/dl, were mildly to moderately azotemic (serum creatinine concentration >1.8 and <5.0 mg/dl), and were free of other systemic disease. The sex of the CRF cats included 6 castrated males and 4 spayed females. The cats in this group ranged in age from 8.2 to 14.3 years (mean, 10.6 years), and the weight ranged from 2.6 to 6.7 kg (mean, 4.2 kg). Nine cats were client-owned and 1 cat was obtained from a research colony.

2.2.2 Study Design

Both groups of cats were treated in an identical manner. Before beginning the study, baseline data including packed cell volume, total plasma protein, urinalysis with culture on urine obtained by cystocentesis, serum ionized calcium concentration, plasma PTH hormone concentration, and serum calcitriol concentration were obtained. The study consisted of 2 phases separated by a washout period. In phase 1, each cat was given calcitriol at a dosage of 2.5 ng/kg q24h PO for 14 days, and in phase 2, each cat was given calcitriol at a dosage of 8.75 ng/kg q84h PO for 14 days. On days 1, 2, and 3 of each phase, serum ionized calcium concentrations were obtained to ensure that ionized hypercalcemia was not developing due to the effects of the drug. Between phase 1 and phase 2 of the study, a 14-day period without any drug administration was included to allow for PTH concentration to return to its pre-study state. On day 14 of each phase, plasma PTH concentrations, and serum concentrations of ionized calcium and calcitriol were obtained before drug administration (time 0) and 2, 4, and 6 h after calcitriol administration.
In the CRF group, serum phosphorus concentrations were controlled with diet in 7 cats, aluminum hydroxide was administered orally as a phosphorus binder in 2 cats or a combination of diet and aluminum hydroxide was used in 4 cats. Three cats were not fed a diet formulated for renal failure due to palatability problems in 2 cats and diet cost in 1 cat.

2.2.3 Sample Analysis:

Throughout the study, blood for plasma PTH concentration determination was collected in EDTA tubes, the samples were separated within 15 minutes of collection, and the plasma was frozen at -80°C until completion of the study. Serum for calcitriol concentration determination was separated within 15 minutes of clot formation, and the serum was frozen at -80°C until completion of the study. All samples were shipped on dry ice to the diagnostic laboratory and were evaluated as batched samples. PTH concentration was measured at The Ohio State University College of Veterinary Medicine and calcitriol concentrations determination was performed at the Michigan State University Diagnostic Laboratory. Serum ionized calcium concentration determination was performed immediately upon collection using a NOVA-7 ion selective electrode. Normal values from these laboratories for PTH are 4-20 pg/ml, 70-100 pmol/L for calcitriol, and 4.8-5.5 mg/dl for ionized calcium.

2.2.4 Statistical Analysis

Descriptive statistics including mean, standard deviation, and range were calculated for age, weight, serum creatinine concentration, plasma PTH concentration, serum calcitriol concentration, and serum ionized calcium concentration for each group of cats during each phase of the study (beginning and end of phase 1, and beginning and end of
phase 2). Analysis for difference of means between groups of cats, throughout both phases of the study, for plasma PTH concentration, serum calcitriol concentration, and serum ionized calcium concentration were performed using one-way ANOVA. A p-value of <0.05 was considered statistically significant.

2.3 Results

All CRF cats remained clinically stable throughout the course of the study. One cat developed a urinary tract infection confirmed by culture and sensitivity on urine obtained by cystocentesis (>30,000 colony forming units/ml urine of mucoid *E. coli*). This cat was treated with amoxicillin for 2 weeks and did not have recurrence of the urinary tract infection. All cats in the normal group remained normal based on physical examination, behavior observed by the owners, and biochemical analysis throughout the duration of the study.

2.3.1 Baseline Data

At the beginning of the study, the serum creatinine concentration for the normal cats ranged from 1.4 to 1.8 mg/dl (mean, 1.6 +/- 0.20) and ranged from 2.2 to 3.9 mg/dl (mean, 2.8 +/- 0.5 mg/dl) in the cats with CRF. The urine specific gravity for the normal cats ranged from 1.042 to 1.069 (mean, 1.058 +/- 0.047) and ranged from 1.015 to 1.024 (mean, 1.017, +/- 0.001) for the cats with CRF. The packed cell volume in the normal cats ranged from 29 to 42% (mean, 34.2 +/- 5.4%) and ranged from 22 to 38% (mean, 32 +/- 5.5%) in the cats with CRF.

2.3.2 Parathyroid Hormone Concentrations

Before beginning phase 1 of the study, the plasma PTH concentration in the normal cats was 2.0 to 31.0 pg/ml (mean, 13.4 +/- 2.7 pg/ml), and was 12.0 to 51.0 pg/ml
(mean, 28.5 +/- 3.9 pg/ml) in the CRF cats. The difference in serum PTH concentration between the groups before beginning the study was statistically significant (p=0.017). At the end of phase 1, the PTH concentration in the normal cats was 2.0 to 19.0 pg/ml (mean, 12.2 +/- 2.0 pg/ml), and was 2.0 to 50.0 pg/ml (mean, 20.9 +/- 2.0 pg/ml) in the CRF cats. The difference in PTH concentration between groups of cats at the end of phase 1 was no longer statistically significant (p=0.29). The difference of plasma PTH concentrations at the beginning and end of phase 1 was statistically significant (p=0.036) in the CRF cats and not statistically significant in the normal cats (p=0.39).

During the 6 hour sampling at the end of phase 1, the mean and standard deviation of plasma PTH concentrations for the normal cats was 10.1 +/- 2.6 pg/ml at time 0, 9.7 +/- 3.2 pg/ml at time 2, 10.1 +/- 3.0 pg/ml at time 4, and 9.6 +/- 2.4 pg/ml at time 6. For the CRF cats, the values were 20.8 +/- 2.2 pg/ml at time 0, 22.9 +/- 3.2 pg/ml at time 2, 23.7 +/- 3.4 pg/ml at time 4, and 17.9 +/- 2.8 pg/ml at time 6. There was no significant difference between or within groups at any time during the 6-hour sampling. The results for the normal and CRF groups for the 6-hour sampling of plasma PTH concentrations in phase 1 and 2 are illustrated in Figure 2.1.

At the beginning of phase 2 of the study, the serum PTH concentration in the normal cats was 2.0 to 42.0 pg/ml (mean, 13.8 +/- 4.8 pg/ml), and 6.0 to 47.0 pg/ml (mean, 23.6 +/- 4.8 pg/ml) in the CRF cats. The difference in plasma PTH concentration between the groups before beginning phase 2 of the study was not statistically significant (p=0.26). At the end of phase 2, the PTH concentration in the normal cats was 2.0 to 48.0 pg/ml (mean, 12.9 +/- 2.0 pg/ml) and 9.0 to 41.0 pg/ml (mean, 20.9 +/- 1.5 pg/ml) in the CRF cats. The difference of plasma PTH
concentrations at the end of phase 2 was not statistically significant (p=0.26) in the CRF group or in the normal cats (p=0.44).

During the 6 hour sampling at the end of phase 2, the mean and standard deviation of plasma PTH concentrations for the normal cats was 8.9 +/- 3.0 pg/ml at time 0, 10.1 +/- 2.8 pg/ml at time 2, 10.2 +/- 3.4 pg/ml at time 4, and 9.7 +/- 2.2 pg/ml at time 6. For the CRF cats, the values were 23.9 +/- 2.2 pg/ml at time 0, 21.2 +/- 3.2 pg/ml at time 2, 16.3 +/- 3.4 pg/ml at time 4, and 18.1 +/- 2.8 pg/ml at time 6. There was no significant difference between or within groups at any time during the 6-hour sampling.

A comparison of the serum PTH concentrations from the beginning and end of both phases of the study is shown in Figure 2.2.

2.3.3 Serum Calcitriol Concentration

Before beginning phase 1 of the study, the serum calcitriol concentration in the normal cats was 52.1 to 286.6 pmol/L (mean, 106.5 +/- 15.8 pmol/L), and was 53.1 to 239.2 pmol/L (mean, 116.0 +/- 21.2 pmol/L) in the CRF cats. At the end of phase 1, the serum calcitriol concentration in the normal group of cats was 234.9 to 286.2 pmol/L (mean, 135.2 +/- 8.9 pmol/L), and was 37.5 to 284.5 pmol/L (mean, 118.9 +/- 7.9 pmol/L) in the CRF cats. There was no significant difference in serum calcitriol concentrations within or between groups during phase 1 of the study.

During the 6 hour sampling at the end of phase 1, the mean and standard deviation of serum calcitriol concentrations for the normal cats was 115.0 +/- 15.5 pmol/L at time 0, 126.3 +/- 17.4 pmol/L at time 2, 139.6 +/- 12.4 pmol/L at time 4, and 156.9 +/- 22.1 pmol/L at time 6. For the CRF cats, the values were 127.7 +/- 12.2 pmol/L at time 0, 137.0 +/- 20.2 pmol/L at time 2, 128.6 +/- 28.4 pmol/L at time 4, and 92.3 +/- 28.8
pmol/L at time 6. There was no significant difference between or within groups at any time during the 6-hour sampling. The results for the normal and CRF groups for the 6-hour sampling of serum calcitriol concentrations in phase 1 are illustrated in Figure 2.3.

At the beginning of phase 2 of the study, the serum calcitriol concentration in the normal cats was 20.1 to 246.6 pmol/L (mean, 114.7 +/- 20.6 pmol/L), and was 57.1 to 190.3 pmol/L (mean, 115.5 +/- 16.7 pmol/L) in the CRF cats. At the end of phase 2, the serum calcitriol concentration in the normal cats was 56.6 to 352.0 pmol/L (mean, 144.1 +/- 8.6 pmol/L), and was 70.0 to 347.4 pmol/L (mean, 142.7 +/- 10.2 pmol/L) in the CRF cats. There was no significant difference in serum calcitriol concentrations within or between groups during phase 2 of the study.

During the 6 hour sampling at the end of phase 2, the mean and standard deviation of serum calcitriol concentrations for the normal cats was 117.0 +/- 19.3 pmol/L at time 0, 163.8 +/- 23.4 pmol/L at time 2, 161.4 +/- 19.4 pmol/L at time 4, and 140.5 +/- 23.9 pmol/L at time 6. For the CRF cats, the values were 146.9 +/- 18.2 pmol/L at time 0, 120.7 +/- 19.2 pmol/L at time 2, 152.0 +/- 20.6 pmol/L at time 4, and 156.2 +/- 37.7 pmol/L at time 6. There was no significant difference between or within groups at any time during the 6-hour sampling. The results for the normal and CRF groups for the 6-hour sampling of serum calcitriol concentrations in phase 1 are illustrated in Figure 2.4.

A comparison of the serum calcitriol concentrations from the beginning and end of both phases of the study is shown in Figure 2.5.

2.3.4 Serum Ionized Calcium Concentration

Serum ionized calcium concentration was measured before and daily for 3 days in both groups of cats during each phase of the study to ensure that ionized hypercalcemia
did not develop. Serum ionized calcium concentration also was measured during each 6 hour sampling in both phases of the study. During the daily sampling of the normal cats in phase 1, the mean and standard deviation of serum ionized calcium concentration was 5.22 +/- 0.06 mg/dl before starting calcitriol, 5.18 +/- 0.03 mg/dl on day 1, 5.13 +/- 0.10 mg/dl on day 2, and 5.08 +/- 0.09 mg/dl on day 3. The mean serum ionized calcium concentrations for the CRF group were 5.19 +/- 0.06 mg/dl before starting calcitriol, 5.25 +/- 0.09 mg/dl on day 1, 5.15 +/- 0.08 mg/dl on day 2, and 5.20 +/- 0.06 mg/dl on day 3. This data is illustrated in Figure 2.6 with individual data points included. At the end of phase 1, after 14 days of therapy, the mean and standard deviation of serum ionized calcium concentration for the normal and CRF group was 5.28 +/- 0.04 mg/dl and 5.10 +/- 0.04 mg/dl, respectively. This data is illustrated in Figure 2.7 with individual data points included.

During the daily sampling of the normal cats in phase 2, the mean and standard deviation of serum ionized calcium concentration was 5.09 +/- 0.05 mg/dl before starting calcitriol, 5.20 +/- 0.05 mg/dl on day 1, 5.12 +/- 0.07 mg/dl on day 2, and 5.12 +/- 0.05 mg/dl on day 3. The mean serum ionized calcium concentrations for the CRF group were 5.04 +/- 0.04 mg/dl before starting calcitriol, 5.08 +/- 0.08 mg/dl on day 1, 5.06 +/- 0.08 mg/dl on day 2, and 5.14 +/- 0.04 mg/dl on day 3. This data is illustrated in Figure 2.6 with individual data points included. At the end of phase 2, after 14 days of therapy, the mean and standard deviation of serum ionized calcium concentration for the normal and CRF group was 5.10 +/- 0.02 mg/dl and 5.12 +/- 0.03 mg/dl, respectively. There was no statistical difference between or within groups at any time.
during the daily serum ionized calcium evaluation in phase 1 or phase 2. This data is illustrated in Figure 2.7 with individual data points included.

2.4 Discussion:

In addition to treatments aimed at increasing quality of life, controlling renal secondary hyperparathyroidism is an important factor in slowing progression and therefore increasing longevity in cats affected with CRF. Traditional treatments include dietary phosphorus restriction alone or in combination with oral phosphorus binders. Controlling serum phosphorus concentration may prevent renal secondary hyperparathyroidism from developing or allow it to be reversed. The use of calcitriol or other vitamin D metabolites as an adjunct in the treatment of renal secondary hyperparathyroidism is routinely used in human medicine and has great potential to benefit veterinary patients. The current study was designed to evaluate the efficacy of calcitriol in treating renal secondary hyperparathyroidism in cats with naturally-occurring CRF, to evaluate for hypercalcemia associated with its administration, to determine the differences in effects of daily and pulse dosage therapy, and to evaluate the effects of dosed calcitriol by evaluating PTH, ionized calcium and calcitriol concentrations before and up to 6 hours after administration.

Before beginning phase 1 of the study, there was a statistically significant difference in PTH concentrations between the normal and CRF cats (p=0.017). At the end of phase 1, this difference was no longer statistically significant (p=0.29) and there was a significant decrease in the PTH concentration within the CRF cats (p=0.036). However, the mean concentration of PTH at the end of phase 1 in CRF (20.9 pg/ml) was higher than the normal cats (12 pg/ml). Differences in PTH values between the
normal and CRF groups before phase 2 (p=0.26) were not statistically significant. This potentially could be explained by an inadequate washout period (14 d) between phase 1 and phase 2 of the study to allow for PTH concentrations in the CRF group to return to their pre-study state. If this is true, 14 d is an inadequate amount of time to allow for re-elevation of PTH concentration. The difference within the CRF group before and at the end of phase 2 also was not statistically significant (p=0.26). However, the end mean PTH concentration (20.9 pg/ml) of the CRF group was identical for phase 1 and phase 2. The end mean PTH concentrations that were obtained, both in phase 1 and phase 2, were dramatically improved when compared to pre-treatment values, but still were slightly higher than the high end of the normal range reported by the laboratory (20 pg/ml). This outcome is considered desirable by human nephrologists. One of the normal cats consistently had PTH values outside the normal range, but this cat did not have an ionized hypercalcemia, enlarged parathyroid glands on ultrasonography, or clinical signs consistent with hyperparathyroidism.

During the 6 h sample collection, the CRF group of cats exhibited a decrease in PTH concentration during both phases of the study. During phase 1, the PTH concentration was 20.8 pg/ml at time 0 and 17.9 pg/ml at time 6. During phase 2, the initial PTH concentration was 23.9 pg/ml and 18.1 pg/ml at time 6. In phase 1, the CRF cats exhibited a transient increase in PTH concentration before a decline was seen. The normal cats had minimal variation in PTH concentrations during the 6 h sampling during both phases of the study. These results suggest that some effects of calcitriol on PTH concentrations appear to be rapid in nature, consistent with transcaltachic effects.
The magnitude of decrease of PTH was not significantly different during the 6 hours in either the daily dosing or pulse dosing phases of the study.

Throughout the study, there was no significant difference between or within groups when evaluating serum calcitriol concentration, as may be expected to occur in the CRF cats with mild to moderate azotemia. At the beginning of phase 1, the mean serum calcitriol concentration for the normal and CRF cats was 106.5 and 116.0 pmol/L, respectively. At the end of phase 1, the serum calcitriol concentration increased to 135.2 and 118.9 pmol/L in the normal and CRF cats, respectively. Pre-treatment calcitriol concentrations in phase 2 for the normal and CRF cats (114.7 and 115.5 pmol/L, respectively) were similar to the pre-treatment concentrations in phase 1. This finding is not unexpected due to the short half-life (4-6 h) of calcitriol. The mean calcitriol at the end of phase 2 were similar in magnitude in the normal cats (144.1 pmol/L) and slightly higher than the CRF group (142.7 pmol/L).

These findings are consistent with a previous study that found low serum calcitriol concentrations in 80% of cats with end stage renal failure, but in only 20% of cats with an earlier stage of renal failure. This finding potentially can be explained by the effect of increased PTH, associated with renal secondary hyperparathyroidism, to stimulate 1α-hydroxylase and promote calcitriol synthesis. In early renal failure, as studied here, adequate renal mass may be present to respond to PTH. As renal failure progresses to end stage, the decrease in functional renal mass may be so great that increases in PTH may have little effect on calcitriol synthesis. Therefore, in early renal failure, calcitriol concentration may be normal or slightly high. This normalization of calcitriol may however lead to a relative rather than absolute calcitriol deficiency due to increased
PTH concentration and potential down regulation of vitamin D receptors in the parathyroid gland.

During the 6 h sampling in both the daily dosage and pulse dosage phases, the serum calcitriol concentration in the CRF cats was higher in the 2 and 4 h samples, and then decreased at the 6 h sample. This result is in contrast to what was observed in the normal group in which calcitriol concentrations were increased in the 2, 4, and 6 h samples above what was observed in the time 0 sample. Serum calcitriol concentration is expected to rapidly normalize due to hepatic clearance in the liver, with some contribution from intestine and cartilage, and inhibition of calcitriol synthesis in the kidney. A potential explanation for this finding may be that the CRF cats have decreased functional renal mass upon which calcitriol acts to inhibit its own synthesis. That is, the serum lowering of calcitriol in the CRF cats may be dependent solely on extrarenal clearance with inhibition of its synthesis within the kidney being less pronounced.

The systemic effects of calcitriol administration primarily include increased gastrointestinal calcium absorption, but mobilization of calcium from bone and reabsorption of calcium from urinary ultrafiltrate also may play important roles. Due to calcitriol’s systemic effects on the gastrointestinal tract, hypercalcemia is the primary adverse effect seen with administration. To evaluate for this effect, the ionized fraction of calcium was monitored closely throughout the course of the study. Serum ionized calcium concentrations remained in the normal range for 9 of the normal cats and 9 of the CRF cats before, during, 3 d after beginning calcitriol therapy, and throughout the 6 h multiple sample collection for both phases of the study. The normal cat that had mild
elevation during the 6 hour sampling and the CRF cat that had a mild elevation 3 days after starting therapy were minor exceptions. These elevations resolved at 8 hours and 4 days respectively. These results suggest that calcitriol, at the dosages used in this study, does not readily cause ionized hypercalcemia. Much higher dosages of calcitriol than those used in this study, however, may lead to increased serum ionized calcium concentration.\(^{61}\)

The results of this study suggest that calcitriol is effective at controlling renal secondary hyperparathyroidism in cats that are mildly to moderately azotemic. Further studies are warranted to evaluate its effectiveness in cats that are more severely affected. Pulse dosing of calcitriol also appears to control renal secondary hyperparathyroidism, but PTH lowering from this type of dosing did not reach statistical significance in the current study. This could potentially be explained by the inadequate washout period after phase 1. Further study is warranted to evaluate the effectiveness of pulse dose calcitriol therapy in cats that have maximally hyperplastic parathyroid glands and circulating PTH hormone. Hypercalcemia was not readily seen in the cats in the current study, and its potential for development with the dosages of calcitriol used here appears to be minimal. Limitations of the current study included an inadequate washout period before phase 2 of the study, and the short duration of each phase of the study. Further effects, either beneficial or adverse, may be seen over a longer period of time. In conclusion, calcitriol has the potential to benefit cats with CRF and its effects should be explored in additional studies.
Figure 2.1: Graphical displays of mean plasma PTH concentration (pg/ml) during 6-hour sampling at the end of phase 1 (q 24 h) and phase 2 (q 84 h) of the study. The CRF group showed decreased serum PTH concentrations at time 6 when compared to time 0. There was no statistical difference between or within groups at any sampling time.
Figure 2.2: Graphical displays of mean plasma PTH concentration in normal and chronic renal failure (CRF) cats on the first (Day 1) and last (Day 14) day of each phase of the study. Statistical significance was seen between the normal and CRF group on day 1 of phase 1 of the study (p=0.017), and within the CRF group on the first and last day of phase 1 (p=0.036).
Figure 2.3: Graphical display of mean serum calcitriol concentrations in normal and CRF cats during 6-hour sampling study at the end of phase 1. There was no statistical difference between or within groups at any sampling time.
**Figure 2.4:** Graphical display of mean serum calcitriol concentrations in normal and CRF cats during 6-hour sampling study at the end of phase 2. There was no statistical difference between or within groups at any sampling time.
Figure 2.5: Graphical display of mean serum calcitriol concentrations in normal and CRF cats on the first (Day 1) and last (Day 14) day of each phase of the study. There were not statistically significant differences of serum calcitriol concentration within or between groups of cats during either phase of the study.
Figure 2.6: Graphical display of mean serum ionized calcium concentrations for both groups of cats during the first 3 days of therapy during phase 1 (q 24 h) and 2 (q 84 h) of the study. An increased serum ionized calcium concentration was not observed in any of the cats during the first 3 days.
Figure 2.7: Graphical display of mean serum ionized calcium concentration (mg/dl) during 6-hour sampling at the end of phase 1 (q 24 h) and phase 2 (q 84 h) of the study. No cat in either group had an elevated serum ionized calcium concentration during the 6-hour sampling in either phase of the study.
BIBLIOGRAPHY


