Use of pamidronate disodium to reduce cholecalciferol-induced toxicosis in dogs

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Objective—To determine whether pamidronate disodium can reduce cholecalciferol-induced toxicosis in a dose-related manner.

Animals—20 clinically normal, 8- to 12-month-old male Beagles.

Procedure—All dogs were given 8 mg of cholecalciferol (CCF)/kg of body weight once orally, then were randomly assigned to 4 groups of 5 dogs each. Dogs were treated with IV administration of 0.9% NaCl solution (SC group), 0.65 mg of pamidronate/kg in 0.9% NaCl solution (LP group), 1.3 mg of pamidronate/kg in 0.9% NaCl solution (MP group), or 2.0 mg of pamidronate/kg in 0.9% NaCl solution (HP group) on days 1 and 4 after administration of CCF. Dogs were observed for 14 days, and serial blood samples were collected for serum biochemical, electrolyte, and 25-hydroxyvitamin D_3 analyses. Urine samples were collected for determination of specific gravity. Glomerular filtration rate (GFR) was determined by plasma iohexol clearance. Histologic examination of renal tissue was performed.

Results—One dog in the SC group was euthanatized 3 days after administration of CCF because of severe clinical signs of toxicosis. Dogs in the HP group had significantly higher mean GFR (day 3), serum potassium concentrations (day 14), and urine specific gravity (days 7 and 14) and significantly lower mean serum creatinine concentrations and total calcium X phosphorus concentration product (days 4 and 7) than dogs in the SC group. Dogs in the HP group had no abnormal findings on histologic examination of renal tissue, dogs in the LP and MP groups had trace to mild mineralization of renal tissue, and dogs in the SC group had moderate mineralization and cellular necrosis of proximal renal tubules.

Conclusions and Clinical Relevance—Pamidronate disodium is a potentially useful drug to reduce CCF-induced toxicosis and other causes of hypercalcemia associated with increased bone resorption in dogs. (*Am J Vet Res* 2000;61:9–13)

Cholecalciferol (CCF) is naturally synthesized in mammalian skin from its precursor, 7-dehydrocholesterol, in the presence of ultraviolet light.[1] The most common cause of vitamin D toxicosis in small animals is accidental ingestion of rodenticides containing CCF as the active ingredient.[2] These products are available in different formulations (ie, granules, flakes, tablets, cakes, or briquettes) containing 0.075% cholecalciferol. Another cause of vitamin D toxicosis in small animals is accidental ingestion of human medications containing vitamin D used for treatment of hypophosphatemic disorders, hypoparathyroidism, osteomalacia, osteoporosis, and renal failure. Recently, ingestion of antipsoriasis petroleum-based creams containing calcipotriol, a congener of 1,25 dihydroxyvitamin D_3, has become a common source of vitamin D toxicosis in dogs.[3] Vitamin D analogues such as 1 α-hydroxyvitamin D_3, 1 α-hydroxyvitamin D_2, dihydrotachysterol, calcitriol, and tacalcitol may have potent calcemic properties and are potentially lethal when ingested at much smaller doses, compared with parent vitamin D compounds. Vitamin D toxicosis in dogs has also been caused by iatrogenic oversupplementation of diets during treatment of hypoparathyroidism.[4]

Vitamin D plays an important role in calcium homeostasis.[4] It enhances calcium and phosphorus absorption from the gastrointestinal tract. In conjunction with parathyroid hormone, it promotes reabsorption of calcium from the distal convoluted tubules of the kidneys and mobilization of calcium from bone. Although the precise mechanisms of bone demineralization are not clear, it is known that vitamin D plays an important role in osteoclastic-mediated bone resorption.[5]

The single oral toxic dose of CCF in dogs is as low as 2 mg/kg of body weight (80,000 U/kg). The median lethal dose (LD_{50}) of CCF in mature dogs is approximately 13 mg/kg.[6] For newer compounds, such as calcipotriol, the single oral toxic dose in dogs is as low as 50 µg/kg.[a] In general, puppies are more susceptible to vitamin D toxicosis than adults, and cats are more sensitive than dogs.[7,8]

Calcitonin is the recommended drug for treatment of CCF-induced hypercalcemia in dogs.[2] Calcitonin by itself is ineffective for treatment of CCF-induced toxicosis in dogs and is always accompanied by aggressive fluid, diuretic, and corticosteroid administration.[9] The half-life of calcitonin is short (3 to 4 hours); therefore, multiple daily injections must be given for ≥ 2 to 3 weeks to maintain blood calcium concentrations within physiologic range.[9,10] Prolonged calcitonin administration also is associated with undesirable adverse effects, such as vomiting and anorexia, and, in humans, a reduced or lack of response after 10 days of treatment has been reported.[11]

Considering the problems associated with calcitonin treatment, we postulated that biphosphonates, a new class of inhibitors of bone resorption, may be beneficial for treatment of CCF toxicosis in dogs. Aminohydroxy-propylidene biphosphonate (pami-

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dronate) is registered in the United States for treatment of hypercalcemia of malignancy and Paget disease in humans.[12,13] We have reported that 1.3 mg of pamidronate/kg can reverse CCF-induced hypercalcemia in dogs.[14] In addition, 2 case reports have indicated that clodronate, a biphosphonate compound closely related to pamidronate, was used successfully for treatment of lymphoma-induced hypercalcemia (1 dog) and vitamin D rodenticide toxicosis (1 dog).[b] The purpose of the study reported here was to determine whether pamidronate can reduce vitamin D_3-induced toxicosis in a dose-related manner in dogs.

Materials and Methods

Twenty 8- to 12-month-old healthy male Beagles were purchased from the Michigan State University Laboratory Animal Resources. Each dog was housed separately in 4.47-m^2 runs. All dogs were acclimated for 7 days before receiving CCF. Temperature and relative humidity were maintained at 17.8 to 28 C and 50 ± 20%, respectively. The dogs were kept on a 12-hour light/dark cycle. Dogs had ad libitum access to food[c] and tap water. Calcium and phosphorus concentrations of the food were 0.9 and 0.8%, respectively. During acclimation, all dogs were determined to be free of parasites. Only dogs considered to be healthy were used in the study. Baseline values of CBC, serum BUN and creatinine concentrations, and urine specific gravity were determined. Following acclimation, all dogs received 8.0 mg of CCF[d]/kg of body weight once orally and were randomly assigned to 4 groups of 5 dogs each. Dogs in the **saline control (SC)** group were given IV infusions of 0.9% NaCl solution (150 ml) during a 2-hour period 24 and 96 hours after CCF administration. Dogs in the **low pamidronate[e] (LP)** group were given IV infusions of 0.65 mg of pamidronate/kg in 150 ml 0.9% NaCl solution during a 2-hour period 24 and 96 hours after CCF administration. Dogs in the **mid-pamidronate (MP)** group received 1.3 mg of pamidronate/kg administered as described for the LP group. Dogs in the **high pamidronate (HP)** group received 2.0 mg of pamidronate/kg administered as described for the LP group. The dose of CCF was selected after a preliminary dose-range study was performed in our laboratory.[14]

Dogs were monitored ≥ 3 times daily for signs of CCF toxicosis and weighed weekly. **Glomerular filtration rate (GFR)** was determined by a plasma iohexol[f] clearance method.[15] Briefly, 1 ml of iohexol (300 mg iodine)/kg was administered IV, and plasma iodine concentrations were monitored by inductively coupled plasma atomic emission spectrometry at 2, 3, and 4 hours. Glomerular filtration rate was calculated by use of a single-compartment model for iodine[15] and was measured 3 times for each dog, during acclimation, at 72 hours after CCF administration (time of peak renal injury), and at the end of the study (day 14).

Blood samples were collected once before CCF administration and on days 1, 4, 7, and 14 after CCF administration for serum biochemical and **25-hydroxyvitamin D_3 (25[OH]D_3)** evaluations. Serum Na, K, Cl, Ca, P, creatinine, and urea concentrations were determined by an autoanalyzer.[g] Serum 25(OH)D_3 was assayed on samples collected on days 0, 1, 4, and 7 after CCF exposure, using a radioimmunoassay.[h] Urine samples were collected, using a urethral catheter, once during acclimation and on days 1, 4, 7, and 14 after CCF administration for determination of specific gravity, using a refractometer.[i]

Fourteen days after CCF administration, surviving dogs were euthanatized by IV administration of 60 mg of pentobarbital sodium/kg. After euthanasia, kidneys were rapidly removed, decapsulated, and weighed. The right kidney was routinely processed for histologic evaluation. All slides were blindly scored by a board-certified pathologist, and morphologic changes were recorded. Mineralization of renal tissue, the major lesion expected in association with hypercalcemia, was graded on a 0 to 3+ scale (corresponding to no, mild, moderate, and severe mineralization).[14]

Statistical analyses were performed, using a computer software program.[j] All data were initially analyzed by repeated-measures ANOVA. When significant differences were detected, statistical comparison between pamidronate-treated groups and the 0.9% NaCl control group was performed by use of the Dunnett multiple-comparison test. A value of $P \leq 0.05$ was considered significant.

Results

All groups of dogs had similar serum concentrations of 25(OH)D_3, a marker of CCF exposure. One dog from the SC group was euthanatized on day 3 after CCF administration because of hematemesis, signs of depression (reduced activity and recumbency), and weakness. All other dogs survived to the end of the study. All dogs from the SC group had clinical signs consistent with CCF toxicosis, including polyuria, polydypsia, anorexia, melena, hematemesis, dehydration, and weight loss. Dogs in the MP and HP groups had the fewest clinical signs, which were mild anorexia and mild (< 10%) weight loss. One dog in the LP group became polyuric, dehydrated, and vomited once on day 2 after CCF exposure. In general, all dogs given pamidronate were alert, playful, and lost significantly less weight, compared with the SC dogs by days 7 and 14 **(Fig 1)**.

Dogs in the LP, MP, and HP groups had significantly lower serum calcium concentrations than the SC group on day 7. In addition, dogs in the HP group had significantly lower serum calcium concentrations than the SC group on days 4 and 14. From days 4 to 14, dogs in the HP group had the lowest serum calcium concentrations. Treatment with pamidronate induced a dose-dependent reduction in total serum calcium concentrations **(Fig 2)**.

Serum phosphorus concentrations (data not shown) are reflected in the product of total serum calcium and phosphorus concentrations. The increase in serum phos-

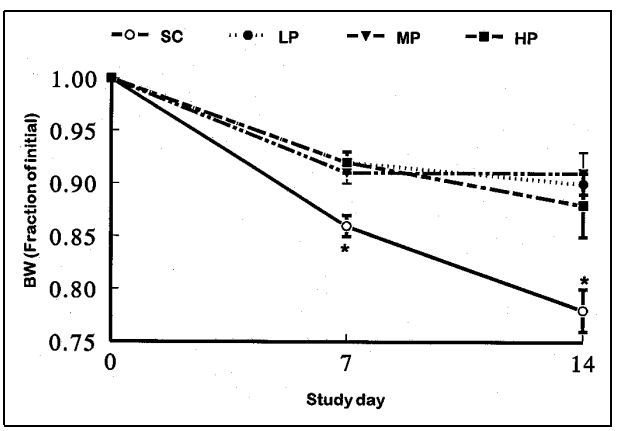

Figure 1—Mean (± SE) changes in body weight (BW, expressed as a fraction of body weight on day 0) in dogs given 8.0 mg of cholecalciferol/kg of body weight (oral) on day 0 and treated with IV administration of 0.9% NaCl solution (SC), 0.65 mg of pamidronate/kg (LP), 1.3 mg of pamidronate/kg (MP), or 2.0 mg of pamidronate/kg (HP) on days 1 and 4. * = Significant difference between control (SC) and treatment (LP, MP, and HP) groups.

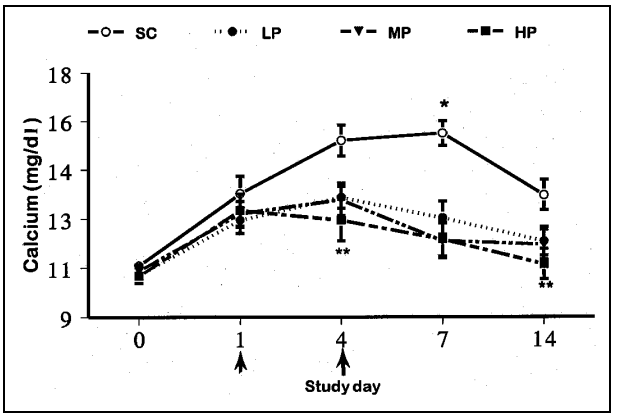

Figure 2—Mean (± SE) total serum calcium concentrations in dogs given cholecalciferol and treated with NaCl solution (SC) or various doses of pamidronate. Arrows indicate days of treatment. * = Significant difference between control (SC) and treatment (LP, MP, and HP) groups. ** = Significant difference between SC and HP groups. *See* Figure 1 for key.

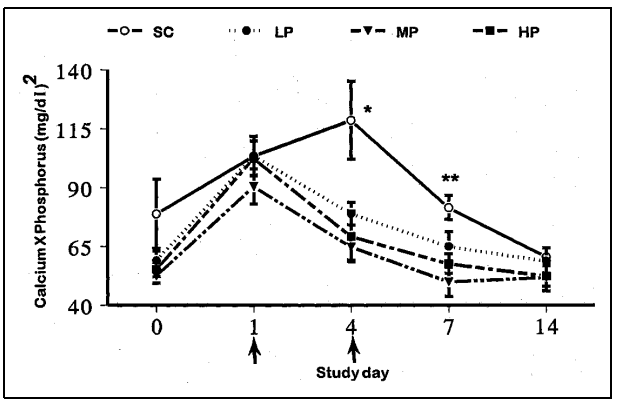

Figure 3—Mean (± SE) of the product of total serum calcium and phosphorus concentrations in dogs given cholecalciferol and treated with NaCl solution or various doses of pamidronate. Arrows indicate days of treatment. * = Significant difference between control (SC) and treatment (LP, MP, and HP) groups. ** = Significant difference between MP and HP groups and control group. *See* Figure 1 for key.

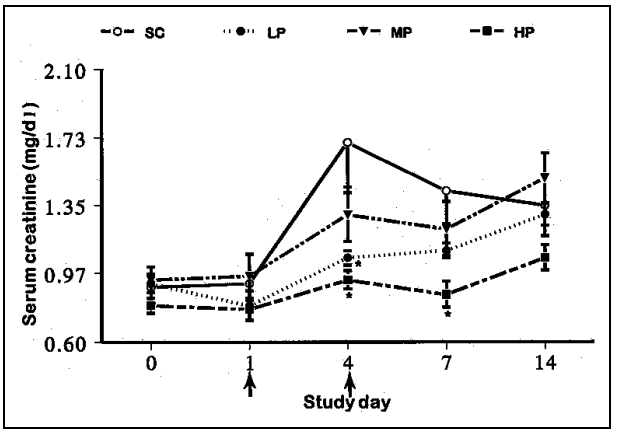

Figure 4—Mean (± SE) serum creatinine concentrations in dogs given cholecalciferol and treated with NaCl solution or various doses of pamidronate. Arrows indicate days of treatment. * = Significant difference between control (SC) and treatment groups (LP, MP, and HP). *See* Figure 1 for key.

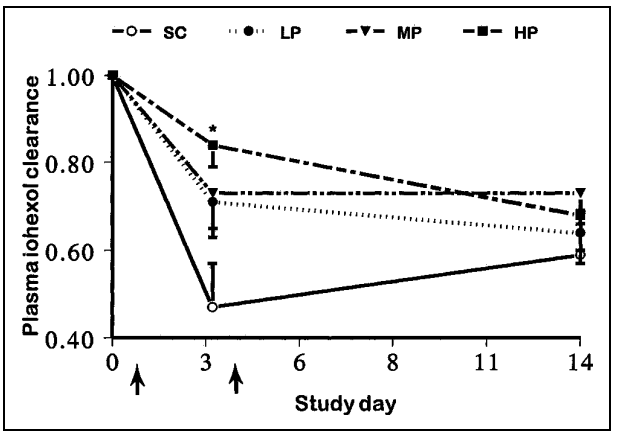

Figure 5—Mean (± SE) plasma iohexol clearance (glomerular filtration rate) expressed as a fraction of initial values in dogs given cholecalciferol and treated with NaCl solution or various doses of pamidronate. * = Significant difference between control (SC) group and HP group. *See* Figure 1 for key.

phorus concentrations paralleled that of serum calcium concentrations. Dogs in the SC group had a significantly higher total serum phosphorus product than all pamidronate-treated dogs on day 4. In addition, dogs in the MP and HP groups had a significantly lower product on day 7, compared with the SC and LP groups (**Fig 3**).

Changes in serum potassium concentrations were also dose-dependent. Significant differences were not detected between treatment groups for serum potassium concentrations. However, dogs in the HP group had significantly higher serum potassium concentrations on day 14 than on day 0. Despite these changes, serum potassium concentrations remained within reference range (4.0 to 5.7 mEq/L) in all dogs throughout the study.

Because the kidney is the primary target organ of CCF toxicosis, **serum urea nitrogen (SUN)** and creatinine concentrations were measured. Dogs in the HP group had significantly lower serum creatinine concentrations, compared with dogs in the SC group on days 4 and 7. On day 4, the LP group also had significantly lower serum creatinine concentrations, compared with the SC group (**Fig 4**). Changes in SUN were similar to those of creatinine (data not shown). All dogs had some reduction in urine specific gravity, but dogs in the HP group excreted significantly more concentrated urine than dogs in the SC group on days 7 and 14.

Glomerular filtration rate, a more sensitive measure of renal function than SUN or serum creatinine concentrations, was decreased approximately 55% on day 3 in the SC group and was significantly lower than the HP group. Dogs in the HP group had a mean reduction in GFR of approximately 15% on day 3. A typical dose-related effect of pamidronate was evident in all treated dogs on day 3. By day 14, GFR was 60 to 85% of initial values in all groups (**Fig 5**).

The most severe histologic lesions in renal tissues were observed in the SC group dogs (**Fig 6**). In this group, mineralization was of moderate severity (2+), with cellular casts in the tubular lumina, especially in the medulla and papillary regions. Trace and minimal (1+) mineralization were observed in the LP and MP groups, respectively. In contrast, histologic lesions were not observed in renal tissues from the HP group (**Fig 7**).

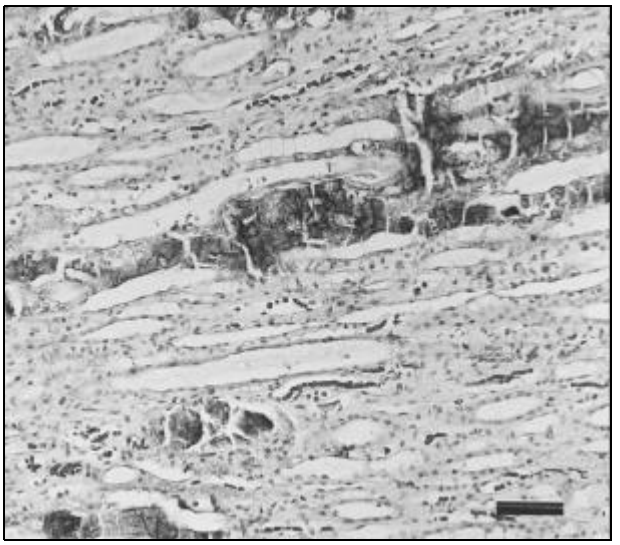

Figure 6—Photomicrograph of a section of renal cortex from a dog given 8.0 mg of cholecalciferol/kg orally and treated with IV administration of 0.9% NaCl solution (control). Notice the moderate amount of mineralization (dark angular material) within medullary tubules. H&E stain. Bar = 70 µm.

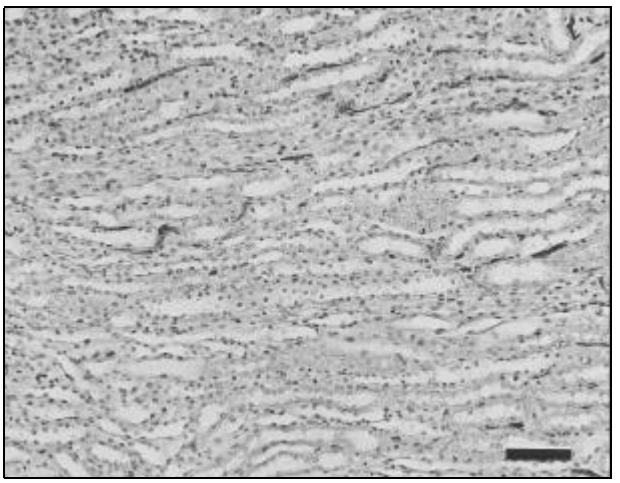

Figure 7—Photomicrograph of a section of renal tissue from a dog given 8.0 mg of cholecalciferol/kg orally and treated with IV administration of 2.0 mg of pamidronate/kg (HP group). Notice the medulla contains no visible mineralization. H&E stain. Bar = 70 µm.

Discussion

Cholecalciferol toxicosis in small animals is a difficult condition to treat, because it has a slow onset and prolonged duration of effects (3 to 4 weeks).[16] Calcitonin, the recommended drug for treatment of CCF toxicosis, has a short half-life of 2 to 3 hours and requires administration of multiple daily injections for ≥ 2 to 3 weeks.[17] Moreover, the efficacy of calcitonin is questionable, and its use is associated with adverse effects (eg, emesis and anorexia).

Because the serum concentration of $25(OH)D_3$ was similar among the 4 groups of dogs, differences observed among treatment groups cannot be explained by differences in CCF exposure; these are likely a result of response to medication. The benefit of pamidronate was evident even in the LP group. Whereas all dogs that received pamidronate survived to the end of the study, 1 dog from the SC group was euthanatized 3 days after CCF administration because of severe clinical signs of toxicosis. Generally, dogs given pamidronate were alert and active. The most obvious clinical sign among dogs given pamidronate was a slight reduction in food intake, which may account for the reduction in body weight observed in these groups (Fig 1). The more severe reduction in body weight observed in the SC group can probably be explained by a number of reasons, including anorexia, polyuria, vomiting, and dehydration.

Hypercalcemia and hyperphosphatemia are the hallmarks of CCF toxicosis in small animals.[2,7,17] Pamidronate, administered at the middle and high doses, was effective in reversing CCF-induced hypercalcemia and hyperphosphatemia. The ability of pamidronate to reduce hypercalcemia in a dose-related manner suggests that this drug has a direct effect on calcium metabolism, most likely by inhibiting bone resorption and decreasing calcium absorption from the gastrointestinal tract.[18] Cellular and molecular mechanisms by which pamidronate reduces hypercalcemia and hyperphosphatemia are not known and deserve further investigation.

Serum potassium concentration was within reference range in all dogs throughout the study, but the data indicated that CCF-induced toxicosis caused a reduction in serum potassium concentration. The potential for hypokalemia in severe cases of CCF-induced toxicosis should not be overlooked. Reasons for the reduction in serum potassium concentration in our study were not determined; however, the role of the kidney in conserving potassium is well known.[19] Reduction in serum potassium concentration may develop as a result of polyuria or from impaired ability of renal tubules to conserve potassium.[16] Reduced food intake may also be a contributing factor. Pamidronate disodium significantly prevented potassium losses, minimizing or perhaps eliminating the need for potassium supplementation, which has been used as an adjunct to calcitonin administration in CCF toxicosis.[16]

Hypercalcemia and hyperphosphatemia were accompanied by reductions in GFR, urine specific gravity, and variable increases in SUN and serum creatinine concentrations. The mechanism of diminished urinary concentrating ability in dogs with CCF toxicosis is not known but may be related to hypercalcemia-induced alterations in renal tubular responsiveness to antidiuretic hormone, alterations in renal medullary solute concentrations, and dystrophic mineralization of renal tubular epithelium. Although a recent report[20] suggests that vitamin D-induced hypercalcemia disrupts urinary concentrating ability by reducing renal medullary content of organic osmolytes, the mechanisms are not understood and deserve further investigation. Azotemia may result from prerenal factors (eg, vomiting, diarrhea, and polyuria) inducing extracellular fluid volume contraction, intrinsic renal factors (hemodynamic or morphologic changes), or a combination of these factors. Of the renal markers monitored in this study, impaired urine concentrating ability was the most persistent effect of CCF toxicosis.

A dose-related response pattern to pamidronate was evidenced by GFR at the time of peak renal injury

from days 3 to 7 (Fig 5). Other factors, such as SUN and serum creatinine concentrations, did not allow for discrimination between the differences in response to the low and middle pamidronate doses. It is interesting that repeated-measures ANOVA did not reveal significant differences among treatment groups, but only 3 data points (days 0, 3, and 14) were examined. The physiologic impact of reduced GFR was evidenced by significant changes that were observed in other markers of renal failure, such as serum creatinine and SUN concentrations. The lack of recovery of GFR after CCF administration, even in the least affected HP group, raises some concern. At euthanasia, the GFR in the MP and HP groups was still 20 to 25% lower than baseline values, although on histologic examination, renal tissues from the HP group appeared microscopically normal. In this and our previous study,[14] we observed higher terminal serum creatinine and SUN concentrations than baseline values among all groups, including those treated with pamidronate. We believe this suggests that CCF toxicosis causes a protracted intrinsic loss of renal function.

Findings from histologic examination were remarkable. Renal tissues from the HP group were microscopically normal, which suggests that tubular necrosis and mineralization were minimal and had reversed by day 14 or had been prevented by pamidronate. Renal lesions in the LP and MP groups were mild. The moderately severe lesions observed in the SC group on day 14 corresponded to the severity of hypercalcemia and increased calcium × phosphorus product in this group. Overall, the histologic findings complimented results of other endpoints and suggested that pamidronate disodium was most effective at the highest dose given to the dogs in this study.

Information on the safety of pamidronate in animals is not available. Information on the drug package insert suggests that increased SUN, serum creatinine concentrations, and renal tubular necrosis developed in dogs given pamidronate in doses ≥ 10 mg/kg. Whereas the benefit of this drug in treatment of CCF toxicosis is clear, clinicians should be aware of the potential of pamidronate-induced toxicosis if administered in high doses. Safety and mechanism of pamidronate-induced nephrotoxicity in dogs are not known and deserve further research.

[a]Volmer P, ASPCA National Animal Poison Control Center, Urbana, Ill: Personal communication, 1998.
[b]Petrie G. Management of hypercalcemia using dichloromethylene biphosphate (clodronate) (abstr), in *Proceedings*. Br Small Anim Vet Assoc Annu Cong 1996:80.
[c]Hill's Science Diet, Hill's Pet Nutrition Inc, Topeka, Kan.
[d]Cholecalciferol, Sigma Chemical Co, St Louis, Mo.
[e]Aredia, Ciba-Geigy Corp, Summit, NJ.
[f]Omnipaque, Nycomed Inc, Princeton, NJ.
[g]Abbott Spectrum, Abbott, Irving, Tex.
[h]Incstar Corp, Stillwater, Minn.
[i]NSG Precision Cells Inc, Farmingdale, NY.
[j]SAS/STAT, version 6, SAS Institute, Cary, NC.

References

1. Host RL, Reinhardt TA. Vitamin D metabolism. In: Feldman D, Glorieux FH, Pike JW, eds. *Vitamin D*. San Diego: Academic Press Inc, 1997;13–31.
2. Dorman DC, Beasley VR. Diagnosis and therapy for cholecalciferol toxicosis. In: Kirk RW, ed. *Current veterinary therapy X: small animal practice*. Philadelphia: WB Saunders Co, 1989;148–152.
3. Fan TM, Simpson KW, Trasti S, et al. Calcipotriol toxicity in a dog. *J Small Anim Pract* 1998;39:581–586.
4. Drazner FH. Hypercalcemia in the dog and cat. *J Am Vet Med Assoc* 1981;178:1252–1256.
5. Suda T, Takahashi N, Abe E. Role of vitamin D in bone resorption. *J Cell Biochem* 1992;49:53–58.
6. Clark ML, Harvey DG, Humphreys DJ. Vitamins. In: Clark ML, Harvey DG, Humphreys DJ, eds. *Veterinary toxicology*. London: Bailliere Tindall, 1981;125–126.
7. Moore FM, Kudisch M, Richter K, et al. Hypercalcemia associated with rodenticide poisoning in three cats. *J Am Vet Med Assoc* 1988;193:1099–1100.
8. Palmer N. Vitamin D poisoning. In: Jubb KVF, Kennedy PC, Palmer N, eds. *Pathology of domestic animals*. 4th ed. Vol 1. San Diego: Academic Press Inc, 1993;81–83.
9. Dorman D, Osweiler GD. Vitamin D toxicity. In: Tilley LP, Smith FWK, MacMurray AC, eds. *The 5 minute veterinary consult*. Baltimore: The Williams & Wilkins Co, 1997;1156–1157.
10. Deftos JL, First PB. Calcitonin as a drug. *Ann Intern Med* 1981;95:192–197.
11. Ober KP. Endocrine agent toxicity. In: Haddad LM, Shannon MW, Winchester JF, eds. *Clinical management of poisoning and drug overdose*. 3rd ed. Philadelphia: WB Saunders Co, 1998;247–264.
12. *Aredia: Pamidronate disodium for injection*. Summit, NJ: CIBA-GEIGY Corp, 1995.
13. Pamidronate. *Med Lett Drugs Ther* 1992;34:1–2.
14. Rumbeiha WK, Kruger JM, Fitzgerald SF, et al. Use of pamidronate to reverse vitamin D_3-induced toxicosis in dogs. *Am J Vet Res* 1999;60:1092–1097.
15. Braselton WE, Stuart KJ, Kruger JM. Measurement of serum iohexol by determination of iodine with inductively coupled plasma-atomic emission spectromentry. *Clin Chem* 1997;43:1429–1435.
16. Fooshee SK, Forrester SD. Hypercalcemia secondary to cholecalciferol rodenticide toxicosis in two dogs. *J Am Vet Med Assoc* 1990;196:1265–1268.
17. Dougherty SA, Center SA, Dzanis DA. Salmon calcitonin as adjunct treatment for vitamin D toxicosis in a dog. *J Am Vet Med Assoc* 1990;196:1269–1272.
18. Gasser AB, Morgan DB, Fleisch H, et al. The influence of two diphosphonates on calcium metabolism in the rat. *Clin Sci (Colch)* 1972;43:31–45.
19. Wright FS, Giebisch G. Regulation of potassium excretion. In: Seldin WD, Giebisch G, eds. *The kidney: physiology and pathophysiology*. Vol 2. New York: Raven Press, 1985;1223–1249.
20. Nakahama H, Nakanishi T, Sugita M. Hypercalcemia reduces renal medullary content of organic osmolytes. *Ren Fail* 1996;18:241–246.