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Research Article

Effect of Dietary Supplements in Reducing Probability of Death for Uremic Crises in Dogs Affected by Chronic Kidney Disease (Masked RCCT)

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Chitosan and alkalinizing agents can decrease morbidity and mortality in humans with chronic kidney disease (CKD). Whether this holds true in dog is not known. Objective of the study was to determine whether a commercial dietary supplement containing chitosan, phosphate binders, and alkalinizing agents (Renal), compared to placebo, reduces mortality rate due to uremic crises in dogs with spontaneous CKD, fed a renal diet (RD). A masked RCCT was performed including 31 azotemic dogs with spontaneous CKD. Dogs enrolled in the study were randomly allocated to receive RD plus placebo (group A; 15 dogs) or RD plus Renal (group B; 16 dogs). During a first 4-week period, all dogs were fed an RD and then randomized and clinically evaluated up to 44 weeks. The effects of dietary supplements on mortality rate due to uremic crises were assessed. At 44 weeks, compared to group A, dogs in group B had approximately 50% lower mortality rate due to uremic crises ($P = 0.015$). Dietary supplementation with chitosan, phosphate binders, and alkalinizing agents, along with an RD, is beneficial in reducing mortality rate in dogs with spontaneous CKD.

1. Introduction

There is a strong consensus to use dietary modification in dogs affected by chronic kidney disease (CKD) [1–7]. Feeding a renal diet (RD) in dogs with mild and moderate spontaneous CKD had beneficial effects on uremia and mortality rate compared to a maintenance diet [1]. In addition, phosphate retention and renal secondary hyperparathyroidism are common complications of CKD [2–7], and hyperphosphatemia is associated with the development of renal lesions in dogs and cats [2–7]. In humans and cats, oral supplementation with compounds such as chitosan (produced by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans and cell wall of fungi), calcium carbonate, and potassium citrate has been advocated to control hyperphosphatemia [4–11]. In addition, chitosan is recognized to reduce azotemia during spontaneous CKD in humans and cats [8–10]. However, whether this holds true for dog has not been previously assessed. Aim of the present study was to evaluate the efficacy of a commercial oral supplement rich in chitosan, enteric phosphate binders, and alkalinizing agents (Renal—Istituto Farmaceutico Candioli SpA, Italy), in reducing mortality rate due to uremic crises in dogs affected by spontaneous CKD, in International Renal Interest Society (IRIS) stages 2, 3, and 4 [4, 12], fed an RD.

2. Materials and Methods

2.1. Animals. Dogs affected by CKD, in IRIS stages 2, 3, and 4, were recruited at the Clinica Veterinaria Pirani of Reggio Emilia, Italy. Results of history, physical examination, including body weight (BW) and body condition score (BCS) (1 to 5 scoring system (3 optimal)), CBC, serum biochemical profile, urinalysis, urine protein-to-creatinine (UPC) ratio, venous blood gas analysis, and indirect blood pressure

measurement were collected. All dogs underwent abdominal ultrasonographic examination, which was performed by the same operator and with the same instrument (Philips HD11XE or Philips HD7XE, Philips Ultrasound, Bothell, Washington, USA). Dogs of any age were included if presenting inactive urine sediment and stable renal function, as defined by serum creatinine concentrations above 1.4 mg/dL (IRIS stages ≥ 2) that did not increase or decrease by 20% or more within 4 weeks from initial determination [4]. In the first 4-week period, all dogs were started on an RD (Royal Canin Renal Canine, Royal Canin SA, Aimargues, France; Hill's Prescription Diet Canine k/d, Hill's Pet Nutrition Inc, Topeka, Kansas, USA).

2.2. Study Design. A randomized, blinded, placebo-controlled clinical trial was performed using a software to allocate cases (MedCalc, Version 11.3.0.0). Informed consent to participate in the study was signed by dog owners.

In the first 4 weeks following inclusion all dogs were started on an RD (Royal Canin Renal Canine, Royal Canin SA, Aimargues, France; Hill's Prescription Diet Canine k/d, Hill's Pet Nutrition Inc, Topeka, Kansas, USA). At the end of this first period, all dogs were clinically reevaluated, performing all above-mentioned laboratory and instrumental analyses and assigned to group A (RD plus placebo), or treatment group B (RD plus Renal). Compositions of the dietary supplements are provided in Table 1.

To mask the identity of the two supplements, they were formulated as powders with identical colours and contained in the same package. After assignment to group A and B, dogs were reassessed between week 4 and 8. Thereafter, examinations were scheduled every 4 months and up to 44 weeks of treatment, or earlier if worsening of clinical signs was noted by the owner.

2.3. Blood Sampling and Assay. During each examination, a blood sample was collected in overnight fasted dogs, and serum was obtained within 30 minutes, stored at 4°C and analyzed within 24 hours. Venous blood gas analysis (Rapidpoint 400, Bayer Health Care, Tarrytown (NY), USA) was immediately performed in all cases.

CBC and serum biochemical analysis, including albumin, total protein, glucose, bilirubin, cholesterol, amylase, alanine transferase, alkaline phosphatase, blood urea nitrogen (BUN), creatinine, ionized calcium, sodium, potassium, chloride, and phosphate, were determined by the use of standard methods (Cobas Mira, Roche Diagnostic, Basel, Switzerland). Blood samples were labelled with alphanumeric codes assigned by randomization to ensure that laboratory personals were blinded during processing. All of the above-mentioned biochemical parameters were used for inclusion and exclusion evaluation.

Table 1: Composition of placebo and Renal.

Placebo	
	Maltodextrin
Renal	
	Maltodextrin
	Calcium carbonate (Ca 38%)
	Potassium Cytrate (K 36%)
	Chitosan

2.4. Urine Sample and Urinalysis. During abdominal ultrasonography, an echo-guided cystocentesis was performed in all dogs, by the use of a 5 mL syringe connected to a 23-gauge needle. All urine samples were put in 10 mL, sterile, evacuated collection tubes labelled with alphanumeric codes based on the previous randomization. All urine samples were analyzed by the same operator. Urines were examined within 60 minutes from collection if samples were stored at room temperature (approx. 20°C), or within 4 hours if samples were stored at 4° to 8°C. Urine sediment was obtained by centrifugation (10 minutes at 900 ×g) of 5 mL of urine, followed by removal of 4.5 mL of supernatant, and by resuspension of the remaining 0.5 mL of urine. A sample of 12 μL of the resuspended urine was microscopically assessed. The supernatant was transferred into separate tubes and stored at −20°C to determine urine protein and creatinine concentration within 7 days. RBCs and WBCs were expressed as mean number of cells/10 hpf (40x magnification). Urine sediment with bacteriuria, and/or >5 RBCs or WBCs/hpf, was considered indicative of active inflammation.

2.5. UPC Ratio. To calculate the UPC ratio, protein concentration (mg/dL) was measured with pyrogallol red, and creatinine (mg/dL) was measured by the use of the Jaffé method in undiluted urine that was thawed before the analysis. Analytes were measured in an automated spectrophotometer (Cobas Mira, Roche Diagnostic, Basel, Switzerland). Dogs were classified as nonproteinuric, borderline proteinuric, or proteinuric according to the IRIS staging system (UPC ratio < 0.2 = nonproteinuric, UPC ratio 0.2 to 0.5 = borderline proteinuric, and UPC ratio > 0.5 = proteinuric) [4, 12].

2.6. Blood Pressure Measurement. Systolic blood pressure measurements were obtained by the use of an ultrasonic Doppler device (DOP 2001, SAMED Elettromedicali srl, Merlino (LO), Italy) in all dogs [13, 14].

2.7. Diagnosis of Uremic Crisis. Diagnosis of uremic crisis was established by clinicians involved in patient management unaware of the supplement being administered. As previously suggested by Jacob and colleagues [1], uremic crisis

Table 2: Mean, median, and 25% and 75% for BW and BCS, hematocrit, serum creatinine, BUN, phosphate, blood pH, bicarbonate, and UPC ratio, for Group A and B at time of randomization. Differences between groups are depicted by P value (statistically significant $P < 0.05$).

	Group A	Group B	P value (A Versus B)
BW (kg)			
median	12.4 *	* 19.8	0.17
25% percentile; 75% percentile	(6.5; 29.0)	(11.7; 31.9)	
BCS (1-5)			
median	3.0	3.0	1.00
25% percentile; 75% percentile	(3.0; 3.0)	(2.0; 3.0)	
Serum Creatinine (mg/dL)			
median	3.1	4.1	0.66
25% percentile; 75% percentile	(1.9; 10.5)	(2.1; 7.23)	
BUN (mg/dL)			
median	69.0	75.1	0.76
25% percentile; 75% percentile	(32.2; 204.0)	(42.9; 150.1)	
Phosphorus (mg/dL)			
median	7.2	7.2	0.90
25% percentile; 75% percentile	(5.4; 11.0)	(5.0; 9.5)	
Blood pH			
median	7.30	7.34	0.55
25% percentile; 75% percentile	(7.25; 7.40)	(7.20; 7.39)	
HCO3− (mEq/L)			
median	19.1	18.2	0.14
25% percentile; 75% percentile	(16.0; 23.4)	(14.6; 22.7)	
Hct (%)			
median	34.00	33.0	0.37
25% percentile; 75% percentile	(24.6; 39.0)	(24.5; 41.0)	
UPC ratio			
median	0.68 *	* 0.39	0.41
25% percentile; 75% percentile	(0.24; 1.60)	(0.29; 0.80)	

was defined when the 3 following findings were observed: (i) identification of at least 2 clinical signs consistent with uremia including depression, lethargy, anorexia, vomiting, uremic breath odour; (ii) serum creatinine concentration at least 20% greater than the previously determined value; (iii) no plausible alternative for these clinical signs.

2.8. Establishing Cause of Death. Causes of death were categorized as nonrenal, probably renal or renal, based on results of anamnesis, physical examination, blood and urine tests, and criteria used to define uremic crisis. To avoid bias, only dogs classified in the third category were considered to have died from a renal event (uremic crisis). Necropsies were not performed in any case.

2.9. Statistical Analysis. Dogs characteristics between groups were compared at the time of group assignment, and intragroup during reexamination at 4–8 weeks of treatment, using the Mann-Whitney nonparametric test. We statistically evaluated the following parameters: BCS and BW, hematocrit, serum creatinine, BUN, phosphate, blood pH, bicarbonate, and UPC ratio.

Kaplan-Meier was used to evaluate the survival probability in both groups and the Logrank test was used to compare rates of death due to uremic crisis between groups. In addition, the Kaplan-Meier was used to evaluate the probability of maintaining stable serum creatinine (serum creatinine concentration not increased above 20% compared to randomization time) in both groups, and the Logrank test