Effects of probiotic VSL#3 on glomerular filtration rate in dogs affected by chronic kidney disease: a pilot study

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Abstract

The aim of the present study was to evaluate the effects of probiotic VSL#3 on glomerular filtration rate in dogs affected by chronic kidney disease.

Treatment group (n=30) received prescription renal diet and VSL#3 (112 to 225×10^9 lyophilized bacteria every 10 kg of BW by mouth SID for 2 months); control group (n=30) received prescription renal diet and standard therapy. All dogs underwent GFR measurement at the beginning of the study (T0). All dogs were re-evaluated by GFR measurement after 2 months (T1).

GFR was significantly higher (p=0.0001) in treatment group compared to control group at T1. In treatment group, GFR was significantly higher (p=0.0008) at T1 compared to T0. In control group, GFR was significantly lower (p=0.001) at T1 compared to T0.

VSL#3 supplementation seemed to be efficient in reducing deterioration of GFR over time in dogs affected by CKD.

Keywords : VSL#3; probiotics; CKD; dog; GFR

Introduction

Uremic retention solutes are generated along the gastrointestinal tract and mostly cleared by the kidneys. Their serum accumulation is negatively correlated with the level of renal function and glomerular sclerosis (1,2). According to recent studies in human medicine (3), the gastrointestinal tract seems to be involved in the pathophysiology of uremic syndrome and contribute to its clinical signs. The effects of probiotics to modulate the intestinal microbiota and to reduce the progression of CKD have been investigated in vitro and in vivo studies in both animals and humans (4).

VSL#3 is a high-dose, multi-strain probiotic product containing viable lyophilized bacteria consisting of 4 strains of Lactobacillus (L. casei, L. plantarum. L. acidophilus, and L. delbrueckii subsp. bulgaricus), 3 strains of Bifidobacterium (B. longum, B. breve, and B. infantis), and 1 strain of Streptococcus sulivarius subsp thermophilus. The VSL#3 strains have shown efficacy in humans for the prevention, treatment, and maintenance of remission of pouchitis and ulcerative colitis (5) it also seems to accelerate gastric ulcer healing (6) and to reduce portal pressure in patients with cirrhosis (7). Recently, VSL#3 has also been used in dogs with idiopathic inflammatory bowel disease (IBD) with promising results (5).

The aim of the present study was to investigate the effects of the administration of VSL#3 on GFR in dogs affected by spontaneous CKD.

Materials and Methods

We included 60 client-owned dogs affected by CKD. Sample size was calculated on the basis of a power analysis with an alpha of 0.05 and power 0.80. There were no restrictions on breed or sex of the dogs. Dogs in IRIS stage 2 and 3 were persistently azotemic, had ultrasound findings consistent with CKD (decreased cortico-medullary distinction) and glomerular filtration rate (GFR) < 60 ml/min/m^2; dogs in IRIS stage 1 had ultrasound findings consistent with CKD (decreased cortico-medullary distinction) and glomerular filtration rate (GFR) < 60 ml/min/m^2 (8). All the patients were classified according to the plasma concentration of creatinine on the basis of the guidelines of the International Renal Interest Society (IRIS). IRIS stage 1 included non-azotemic dogs (creatinine < 1.4 mg/dl), with ultrasound findings consistent with CKD, inadequate urinary concentrating ability (USG<1030), and GFR < 60 ml/min/m^2. Patients were considered proteinuric if they were found repeatedly with a UPC ≥ 0.5 in 3 or more specimens, obtained 2 or more weeks apart. IRIS 1 dogs, which met the inclusion criteria for CKD IRIS stage 1, but with a USG>1030, were considered eligible for the study if they had protein-losing nephropathy. Animals were excluded from the study if they were in IRIS stage 4 (creatinine > 5 mg/dl) for ethical reason. Dogs with evidence of acute kidney injury (AKI) or other significant systemic or organ-related disease, such as, neoplastic, cardiovascular, liver and gastrointestinal disease, assessed by clinical and ultrasound evaluation and serum biochemistry were not included in the study. CKD dogs with evidence of positive urine culture were excluded from the study. After the full workup 24 dogs in IRIS stage 1, 16 dogs in IRIS stage 2 and 20 dogs in IRIS stage 3 were considered eligible for the study.

Dogs with persistent proteinuria (n=32) were treated with benazepril (0.25-0.5 mg/kg once to twice daily). Dogs with vomiting and/or poor appetite (n=9) were treated with maropitant (1 mg/kg once daily) and ranitidine (2 mg/kg twice daily). Dogs showing hypo-proliferative anemia (n=4) with

HCT<20% were treated with darbopoetin-alpha (0.5-1 μcg/kg once weekly). Dogs (n=20) with a history of hypertension (BP >160 mmHg) were maintained on a combination of benazepril and amlodipine (0.25-0.5 mg/kg once daily). Dogs with serum phosphate > 5 mg/dl (n=10) were treated with aluminium hydroxide (50-100 mg/kg daily). Dogs in IRIS stage 2 and 3 with clinical signs (vomiting, poor appetite) and/or proteinuria, anemia, hypertension, hyperphosphatemia were started on appropriate treatment weeks to months prior to T0. These drugs were continued during the study period.

On the day of enrolment (T0) 12 of the 24 dogs with IRIS stage 1, 8 of the 16 dogs with IRIS stage 2, and 10 of 20 the dogs with IRIS stage 3 were randomized into two groups (control group and treatment group) using a computer-generated randomization list. Control group (CG) was composed by 30 dogs (IRIS stage 1 n= 12; IRIS stage 2 n= 8; IRIS stage 3 n=10). Treatment group (TG) was composed by 30 dogs (IRIS stage 1 n= 12; IRIS stage 2 n= 8; IRIS stage 3 n=10). Dogs of treatment group (TG) received VSL#3 at the dose of 112 to 225×10^9 lyophilized bacteria every 10 kg of body weight by mouth SID for 60 days (5), in addition to the ongoing therapy. After randomization, patients of both groups were submitted to GFR evaluation through the plasma clearance of iohexol (8), evaluation of serum creatinine, urea, phosphate, complete urinalysis and UPC, urine culture and blood pressure monitoring (PetMAP-Ramsey Medical Inc, Tampa, FL, USA). For blood pressure a mean of five consecutive measurements was considered. Hydration status of patients was assessed before blood sampling and GFR evaluation, in order to be sure they were not dehydrated. None of the dogs was dehydrated at time of blood sample and GFR determination. Data were recorded as T0. For both groups, GFR, serum creatinine, urea, phosphate, complete urinalysis and UPC, blood pressure and urine culture were reassessed at T1. This study was conducted in a single-blinded manner. To keep the investigator blinded to the study, a dispenser was used to supply VSL#3, according to a

predetermined randomisation code. Each owner was instructed not to mention VSL#3 at the time of the recheck. The study was approved by the University of Pisa committee.

Statistical analysis was conducted with commercial available software (GraphPad Prism®-Software, Inc., La Jolla, CA, USA). Data were tested for normality with D'Agostino and Pearson test. Data were non-normally distributed and they were presented as median (min.-max.). Differences between groups were assessed using a Wilcoxon signed rank test. A level of $P \leq 0.05$ was considered significant for all tests.

Results

At baseline (T0) in CG, 14/30 dogs were proteinuric (UPC>0.5) and 4/30 dogs were borderline proteinuric (UPC>0.2 and <0.5); 1 dog was severely hypertensive (BP >180 mmHg) and 3/30 dogs were moderately hypertensive (BP>160 mmHg and <180 mmHg). In TG, 18/30 dogs were proteinuric (UPC>0.5) and 3/30 dogs were borderline proteinuric (UPC>0.2 and <0.5); 4/30 dogs were moderately hypertensive (BP>160 mmHg and <180 mmHg). At T0 there were no significant differences in age, weight, GFR, serum creatinine, urea, phosphate, blood pressure, UPC and USG between dogs of CG and TG (Table 1). In CG 11/30 dogs were on a combination of benazepril and amlodipine to control blood pressure. In TG 9/30 dogs were on a combination of benazepril and amlodipine to control blood pressure. In dogs of CG, GFR was lower (P = 0.0002), and creatinine and USG higher (P = 0.001 and P = 0.04 respectively) at T1 compared with T0. No significant difference was observed in urea, phosphate, blood pressure and UPC between T0 and T1. At T1, 24/30 dogs of CG were proteinuric and 2/30 were borderline proteinuric; while 15/30 dogs of TG were proteinuric and 3/30 were borderline proteinuric. No significant difference in the number of proteinuric and non proteinuric dogs was found between CG and TG (p=0.18). When only proteinuric dogs (n=14) of CG are considered, no significant difference in UPC was found between T0 and T1. In dogs of TG, GFR and USG were higher (P = 0.001 and P = 0.0001 respectively), and UPC lower (P = 0.006) at T1 compared with T0. When only proteinuric dogs (n=18) of TG are considered, a significant reduction (p=0.06) in UPC was found at T1. At T1, 4/30 dogs of CG were moderately hypertensive; while 2/30 dogs of TG were moderately hypertensive and 1 dog was severely hypertensive. No significant difference was observed in creatinine, urea, phosphate, and blood pressure between T0 and T1. Values of GFR, creatinine, urea, phosphate, blood pressure, UPC and USG for CG and TG at T0 and T1 are reported in Table 2.

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Discussion

In this study we determined that GFR, measured through the plasma clearance of iohexol, is increased in dogs treated with VSL#3, when compared with dogs treated with standard therapy (Figure 1). Our findings seemed to be in agreement with previous results (9), in which the group of patients on probiotic and prebiotic supplementation showed a significantly reduced decline of GFR over time, compared with the group of patients on protein-restricted diet only (9). In our study, patients on prescription renal diet only showed a significant reduction of GFR over time. This finding may be due to an incomplete ability of prescription renal diet to block the production of uremic retention solutes. Koppe and Collegues (4) postulated that the production of uremic retention solutes, mainly generated by protein degradation, cannot be completely blocked by a low-protein diet, and modelling intestinal microbiota can be considered as an additional beneficial intervention (4). The reason for using probiotics during CKD is to enhance the intestinal removal of uremic retention solutes. A food-grade, gram-positive bacteria, in a probiotic formulation, was previously found to be beneficial to rodents (10), miniature pigs (11) and cats (12) with renal failure. Ranganathan et al, reported that probiotic dietary supplements facilitated the reduction of blood concentrations of uremic toxins, reduced the progression of renal impairment, and prolonged survival in rat models with CKD (13). In one report (14) the use of probiotics (in particular Kibow biotics) in 2 uremic dogs showed favourable and encouraging results, while Polzin (15) did not find any significant difference between 32 CKD dogs treated with Azodyl® (Vetoquinol) vs placebo. In human medicine, CKD has been associated with alterations of the gastro-intestinal mucosa and disequilibrium in the intestinal flora. This condition is responsible for an increased transformation of amino acids into uremic retention solutes (16). Elevated serum concentrations of indoxyl-sulfate, p-cresyl sulfate and trimethylamine n-oxide were negatively

correlated with the level of kidney function and predictors of CKD progression (1). These uremic toxins would be responsible for a worsening of renal function by different mechanisms. One study in experimental rats suggested that elevated serum level of uremic toxins may accelerate the onset of kidney tubular damage. In nephrectomized rats, GFR was significantly lower in rats treated with uremic toxins compared with controls. The reduction in GFR correlated with a higher glomerular sclerosis, which was promoted by the elevated levels of uremic toxins (17). In a previous study by Miyazaki and Collegues (2), the administration of indoxyl-sulfate to uremic rats mediates the kidney expression of genes related to tubule-interstitial fibrosis and it is associated with significant decline in renal function and worsening of glomerular function (2). Elevated levels of indoxyl sulfate were also associated with vascular stiffness, aortic calcifications and high cardio-vascular mortality in humans affected by CKD (18).

If we compare the serum values of creatinine and urea of the two groups of patients, we notice that no significant difference was found for the treatment group between T0 and T1, while the control group showed a significant increase in serum creatinine at T1. Although in CG the increase in serum creatinine seems to reflect an actual worsening of renal function, in TG both creatinine and urea showed only a non-significant trend to reduce at T1, compared with T0, despite a significant improvement in GFR. This finding was not unexpected. Serum creatinine and urea are generally used as indirect markers of renal function, but they may be affected by extra-renal factors. We opted to measure GFR, as it is universally considered the gold standard test to assess overall renal function (19). It is also possible that the trend to reduce of creatinine and urea in VSL#3 group may be due to a direct degradation of VSL#3. VSL#3 contains, among others, Lactobacillus delbrueckii, which has been reported to hydrolyze urea in vitro (13). Therefore reduction in serum levels of urea and creatinine, in patients treated with probiotics, should be evaluated carefully, as it may not reflect an actual improvement of kidney function (4).

The improvement of GFR in the VSL#3 group was also accompanied by a significant increase of USG and reduction of UPC at T1. In dogs of CG, USG was significantly reduced at T1, compared with T0, while UPC showed only a non-significant trend to reduce. These findings may reflect an overall improvement of kidney function in patients treated with VSL#3. A recent study (20) reported that supplementation of Lacobacillus species in rats with CKD reduced systemic inflammation and proteinuria, playing a protective role in reducing the progression of CKD (20).

The present study shows a few limitations. As we had no clear evidences of potential benefits of VSL#3 in controlling clinical signs of CKD and reducing the progression of the disease, we did not consider ethical to enrol dogs with IRIS stage 4 and/or end-stage renal disease. As a consequence, we have no data regarding the effects of VSL#3 on GFR in these two populations. As the relatively low number of patients enrolled in the study, we opted to consider all CKD patients together. A larger study would be recommended to compare the effects of VSL#3 on GFR in dogs at different stages of CKD, in order to verify if the severity of CKD may or may not affect the efficacy of VSL#3. Another limitation of the present study is represented by the fact that during the study period 20/60 dogs were on a combination of benazepril and amlodipine to control blood pressure. Although no randomization for blood pressure was done prior to T0, the number of dogs on benazepril and amlodipine was almost equal in both CG (n=11) and TG (n=9). However, the Authors cannot exclude that the concomitant use of benazepril and amlodipine in association with VSL#3 might contribute to improve UPC at T1 in this group of patients. It has also to be noticed that the slight higher number of hypertensive dogs in CG might affect the progression of CKD and contribute to the worsening of GFR and UPC at T1.

In conclusion the administration of VSL#3 at the dose of 112 to 225×10^9 lyophilized bacteria every 10 kg of body weight by mouth SID for 60 days seemed to affect significantly GFR, USG and UPC in CKD dogs. After two months of VSL#3 supplementation, treated dogs showed a significant improvement of GFR and USG and a significant reduction of UPC compared to control dogs. Our

findings seemed to support a potential role of VSL#3 in reducing the progression of CKD in dogs. Results from this pilot study should encourage a larger study.

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Table 1: signalment and baseline values (T0) of GFR, serum creatinine, urea, phosphate, blood pressure, UPC and USG of dogs of CG and TG

	CG (n = 30)	TG (n = 30)	P value
Age (years)	5.2 (1-12)	6.8 (1-13)	0.32
Body weight (kg)	28.5 (7.4-72)	29.3 (8-72)	0.51
GFR (ml/min/m^2)	37 (12-59)	40 (15-56)	0.23
Creatinine (mg/dl)	1.8 (0.9-2.4)	1.4 (0.9-3)	0.63
Urea (mg/dl)	64 (27-157)	58 (18-112)	0.47
Phosphate (mg/dl)	4.3 (2.8-6.8)	4.1 (2.9-9.7)	0.78
BP	128 (115-189)	130 (115-176)	0.98
UPC	0.9 (0.08-7.5)	1.29 (0.13-4.94)	0.89
USG	1016 (1005-1046)	1015 (1002-1038)	0.31

Data were non-normally distributed and presented as median (min.-max.). P≤0.05 was considered significant. BP: blood pressure

Table 2: Values of GFR, serum creatinine, urea, phosphate, blood pressure, UPC and USG of dogs of CG and TG at T0 and T1

	CG (n = 30)		
	T0	**T1**	**P value**
GFR (ml/min/m^2)	37 (12-59)	30 (10-47)	**0.0002**
Creatinine (mg/dl)	1.7 (0.9-5.9)	1.8 (0.9-2.4)	**0.001**
Urea (mg/dl)	64 (27-157)	62 (23-250)	0.28
Phosphate (mg/dl)	4.3 (2.8-6.8)	5.3 (2.6-14.8)	0.27
BP	128 (115-189)	127 (115-170)	0.58
UPC	0.9 (0.08-7.5)	1.2 (0.1-9.3)	**0.04**
USG	1016 (1005-1046)	1012 (1005-1048)	**0.04**
	TG (n = 30)		
	T0	**T1**	**P value**
GFR	40 (15-56)	48 (11-75)	**0.001**
Creatinine	1.4 (0.9-3)	1.4 (0.9-4.7)	0.87
Urea	58 (18-112)	51 (18-153)	0.10
Phosphate	4.1 (2.9-9.7)	3.85 (3.40-11.2)	0.85
BP	130 (115-176)	130 (115-185)	0.68
UPC	1.29 (0.13-4.94)	0.77 (0.09-3.76)	**0.006**
USG	1015 (1002-1038)	1018 (1010-1047)	**0.0001**

Data were non-normally distributed and presented as median (min.-max.). P≤0.05 was considered significant. BP: blood pressure

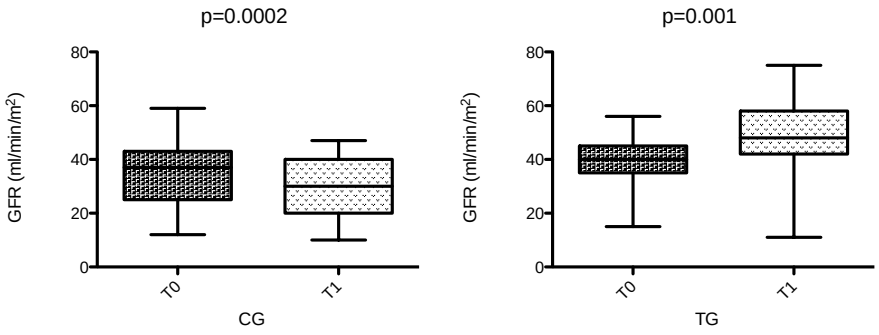

Figure 1. Wilcoxon test ($p<0.05$) between GFR values at T0 (baseline) and T1 (after 2 months) in dogs of control group (n=30) and in dogs of treatment group (n=30). In dogs of control group GFR was lower ($p=0.0002$) at T1 compared with T0. In dogs of treatment group GFR was higher at T1 ($p=0.001$) compared with T0.

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