http://dx.doi.org/10.1590/1678-4162-12392

Arq. Bras. Med. Vet. Zootec., v.74, n.2, p.310-318, 2022

Effects of intravenous administration of allogeneic mesenchymal stromal cells, derived from adipose tissue, in five dogs with chronic kidney disease

[Efeitos da administração intravenosa de células mesenquimais alogênicas, derivadas do tecido adiposo, em cinco cães com doença renal crônica]

M. *Milistetd* [1], C.Z. *Cavalcante*[2], H.S.S. *Brunel*[3], L.M.B. *Leite*[4], P.E. *Mosko*[5], P. F. *Malard* [3], P.V. *Michelotto Júnior*[2]

[1]Graduate, Pontifícia Universidade Católica do Paraná, Curitiba, PR, Brasil
[2]Pontifícia Universidade Católica do Paraná, Curitiba, PR, Brasil
[3]Practitioner, Brasília, DF, Brasil
[4]Biologist, Pontifícia Universidade Católica do Paraná, Curitiba, PR, Brasil
[5]Universidade Positivo, Curitiba, PR, Brasil

ABSTRACT

This study aimed to evaluate the safety of allogeneic adipose-derived mesenchymal stromal cell (aASC) treatment in dogs with chronic kidney disease (CKD) at the time of infusions and during the 120-day follow-up after the last infusion. Five dogs with CKD received three intravenous infusions of approximately $1\times10^6\pm10\%$ of aASCs per kilogram of body weight at 21-day intervals. Clinical and laboratory evaluations were performed at the time of each treatment and at 30 and 120 days after the last infusion. Adverse effects of the treatment were assessed using clinical observations, laboratory analyses, and owners' answers about their dog's behavior after infusions and during follow-up. The investigated animals did not present any adverse effects immediately after infusion or during the follow-up after the last infusion according to clinical and laboratory observations, as well as the dog owner's descriptions. One treated animal showed a reduction in creatinine, from 3.5mg/dL to 2.4mg/dL from day 0 to day 153, gained 100g of body weight, and improved disposition. The study results demonstrate that aASC therapy is safe for dogs with CKD; however, further studies are needed to investigate these promising results.

Keywords: dog, kidney disease, quality of life, cell therapy, safety

ABSTRACT

Este estudo teve como objetivo avaliar a segurança do tratamento com células estromais mesenquimais alogênicas derivadas do tecido adiposo (aASC) em cães com doença renal crônica (CKD) no momento das infusões e durante o acompanhamento de 120 dias após a última infusão. Cinco cães com CKD receberam três infusões intravenosas de aproximadamente $1\times10^6\pm10\%$ de aASC por quilograma de peso corporal em intervalos de 21 dias. Foram realizadas avaliações clínicas e laboratoriais no momento de cada tratamento e aos 30 e 120 dias após a última infusão. Os efeitos adversos do tratamento foram avaliados utilizando observações clínicas, análises laboratoriais e respostas dos proprietários sobre o comportamento de seu cão após as infusões e durante o acompanhamento. Os animais investigados não apresentaram nenhum efeito adverso imediatamente após a infusão ou durante o acompanhamento após a última infusão de acordo com as observações clínicas e laboratoriais, bem como as descrições do dono do cão. Um animal tratado apresentou uma redução na creatinina, de 3,5mg/dL para 2,4mg/dL do dia 0 ao dia 153, ganhou 100g de peso corporal, e melhorou a disposição. Os resultados do estudo demonstram que a terapia aASC é segura para cães com CKD; entretanto, são necessários mais estudos para investigar estes resultados promissores.

Palavras-chave: cão, doença renal, qualidade de vida, terapia celular, segurança

Corresponding author: michelemilistetd@hotmail.com
Submitted: April 13, 2021. Accepted: December 20, 2021.

INTRODUCTION

Chronic kidney disease (CKD) is an irreversible, progressive disease that often affects approximately 25% of dogs (Lund *et al*., 1999; Pelander *et al*., 2015). It is characterized by a structural or functional abnormality of the kidneys, leading to the loss of renal function over the course of at least 3 months (Polzin, 2011).

Although CKD is chronic, it causes a constant inflammatory state in the patient, which, together with oxidative stress, leads to several serious consequences, such as anemia, nutritional changes, and endothelial and immunological dysfunction (Kogika *et al*., 2015).

Clinical signs are usually nonspecific and are observed in more advanced stages of the disease. The most common manifestations are polyuria, polydipsia, weight loss, poor coat quality, halitosis, stomatitis, gastroenteritis, and dehydration (Cowgill *et al*., 2016).

Treatment must be targeted at each stage of the disease; however, adaptations in therapy must accompany an individual clinical response. Treatment has two main goals: to slow the progression of the disease, preserve functional nephrons, and reduce clinical signs; and to improve the patient's quality of life. Among the main guidelines for treatment are discontinuing the use of nephrotoxic drugs; identifying and treating pre- and post-renal abnormalities; using specific diets; maintaining hydration; and treating urinary tract infections, hypertension, proteinuria, hyperphosphatemia, metabolic acidosis, and anemia (Iris, 2019).

For kidney diseases, like with many other chronic diseases, the use of cell therapies has been proposed. The main objective of this therapeutic modality for patients with kidney disease is to improve their quality of life and delay disease progression. These goals are achieved through the administration of mesenchymal stromal cells (MSCs), as they can reduce local inflammation in different organs and tissues (Sutton *et al.*, 2002).

MSCs obtained from adipose tissue seem to be safe for allogeneic application without the risk of rejection by the recipient's immune system, as these cells appear to have a low degree of expression of class I major histocompatibility complex (MHC I) and no expression of MHC II on the cell surface (Le Blanc *et al*., 2003). Thus, it is possible to use allogeneic MSCs from a cell bank in urgent cases of application, such as in cases of acute renal failure or in the exacerbation of chronic renal disease.

In this context, cell therapy using MSCs has been studied as a possible treatment for kidney disease. Recently, Villanueva *et al*. (2019) evaluated the efficacy and safety of intravenous infusion of autologous adipose tissue-derived mesenchymal stromal cells (ASCs) for the treatment of CKD in six human patients using an application protocol of 1×10^6 cells/kg of body mass. They did not find any adverse effects or possible benefits of ASC therapy in these patients.

Similarly, Quimby *et al*. (2011) infused autologous ASCs in cats at three points in the renal cortex under ultrasound guidance; they observed a slight decrease in serum creatinine levels, but there were no adverse effects. In a study by Thomson *et al*. (2019), autologous MSCs were infused into cats via the renal artery. The study demonstrated a possible route of administration and reported that there were no adverse effects 3 months after treatment.

In another study, Quimby *et al*. (2013) investigated intravenous infusion of 2 and 4×10^6 cryopreserved allogeneic ASCs and 4×10^6 cells cultured from cryopreserved adipose, every two weeks (total three infusions). Cats receiving a lower dose of ASC showed no adverse effects. However, there was no clinical improvement after treatment, suggesting that the effects of cell therapy should be evaluated in the medium- and long-term. The treatments caused immediate adverse effects in the treated animals receiving the higher dose, such as vomiting and increased respiratory rate.

However, studies using MSCs in dogs with CKD are scarce and there is no information on its use in terms of safety, possible effects on kidney function, and quality of life in dogs with CKD. Therefore, considering the therapeutic potential of MSC treatment and the lack of studies proving its safety in CKD dogs, this study aimed to evaluate the safety of intravenous infusion of allogeneic ASCs (aASCs) in dogs with CKD

immediately after each infusion and during 120 days of follow-up after the last infusion.

MATERIALS AND METHODS

This prospective longitudinal clinical study was approved by the Ethics Committee on Animal Use of the Pontifícia Universidade Católica do Paraná (PUCPR), registered under number 01214/2017.

Five dogs of different breeds, sex, and age diagnosed with stage I or stage II CKD during routine clinical practice in two private clinics in Curitiba, Brazil, were investigated (Table 1). CKD diagnosis was first performed by private veterinary clinicians who then referred dogs for participation in this study. CKD was staged according to the guidelines proposed by the IRIS (2019). Inclusion criteria included dogs who were not administered analgesics or anti-inflammatory drugs immediately before or throughout the study period. Dog owners were informed of the study. After obtaining signed consent, the patients were administered cell therapy concomitantly with the clinical therapy previously established by the veterinarians responsible for the dogs.

Table 1. Breed, age, and IRIS stage of dogs with chronic kidney disease treated with allogeneic adipose tissue-derived mesenchymal stromal cells

Dog	Breed	Description	IRIS stage *
1	Pinscher	16 YO gelding	II
2	Mixed breed	15 YO gelding	I
3	Mixed breed	8 YO gelding	II
4	Mixed breed	13 YO gelding	II
5	Jack Russel terrier	11 YO gelding	II

YO, years old; IRIS, International Renal Interest Society. *IRIS stage on admission to the study.

All investigated animals received three infusions of aASCs at 21-day intervals between consecutive infusions on days 0, 21, and 42. Clinical and laboratory evaluations were performed at the time of each infusion, as well as 30 and 120 days after the last infusion.

During the clinical evaluation, the owners were asked about clinical evolution, use of medications, exposure to toxins, appetite and type of feeding, behavior and urinary frequency, volume and color of the urine, and quantity and quality of water ingested. Physical examination included the evaluation of the state of consciousness, body mass, hydration status, mucosal membranes, peripheral lymph nodes, heart rate, respiratory rate, and rectal temperature. Systolic blood pressure (SBP) was measured using a model 811 B Doppler device (Parks Medical, USA).

For laboratory evaluations, venous blood was collected from the jugular vein and was immediately analyzed at the Laboratório de Análises Clínicas – Clinilab (Curitiba, Brazil) to evaluate the total erythrocyte and leukocyte counts, serum urea level, creatinine level, phosphorus level, symmetrical dimethylarginine (SDMA) level, potassium level, sodium level, ionic calcium level, and blood gas analysis.

Urine was collected using ultrasound-guided cystocentesis (Chew *et al.*, 2011). Urinalysis was performed, and the urine protein-to-creatinine ratio (UPC) was calculated at the Laboratório de Análises Clínicas – Clinilab (Curitiba, Brazil).

Subcutaneous adipose tissue was collected from three healthy dogs from the region lateral to the origin of the tail (approximately 20 g). The culture and characterization of MSCs were performed as described by Falcão *et al.* (2020). aASCs were frozen in 0.5mL straws at a concentration of 1.25 million cells per straw in a freezing solution containing dimethylsulfoxide solution and fetal bovine serum. The obtained aASCs were further thawed and tested for the presence of mycoplasma, fungus, and bacteria using polymerase chain reaction (PCR) (VeritiThermalCycler, Thermo Fischer Scientific). The aASCs were then frozen in a nitrogen canister until the day of infusion. On the

day of treatment, the cells were thawed and washed to remove the freezing solution. After washing, the cells were diluted in transport solution, stored in a syringe, and prepared for infusion.

All patients involved in this study received an infusion of $1\times10^6 \pm 10\%$ ASCs per kg of body mass, diluted in 50 mL of lactated Ringer's solution (Fresenius Kabi Brasil Ltda., Brazil), given intravenously via the cephalic vein for 30–40 minutes; each patient randomly received cells from one of the three donors. Infusions were performed on days 0, 21, and 42.

Some of the cells used were also analyzed for cell viability. For this, we used the vital dye 7-AAD (BD Pharmingen, USA) for 30 min, washed with PBS (Gibco Invitrogen, USA), and fixed with PBS containing 1% paraformaldehyde (Sigma Aldrich, Brazil). The samples were acquired using a FACSCalibur flow cytometer (BD Bioscience, USA) and analyzed using FlowJo software (FlowJo, Ashland, USA); the results are described as the percentage of dead cells.

The data analysis was initially descriptive. The clinical and diagnostic (parametric) data collected in the study were analyzed for normality using the Shapiro–Wilk test, and then described as mean and standard deviation. The laboratory results of each moment were analyzed using repeated measures analysis of variance followed by the post hoc Bonferroni test, considering the significance level at 5% and p-value >0.05, using the STATA software (version 14, College Station, Texas, USA).

RESULTS

Three samples of cryopreserved aASCs analyzed for cell viability demonstrated 8.31%, 7.82%, and 6.65% dead cells, respectively, indicating a viability of >90% after thawing.

According to the dog's tutors' descriptions, none of the patients showed any adverse effects. There were no episodes of vomiting, diarrhea, fever, change in respiratory and/or heart rate, or anaphylactic reaction during and 1 hour after each infusion. In addition, there were no changes, such as apathy or lack of appetite, after aASC infusion.

The results related to body weight, SBP, total erythrocyte and blood leukocyte counts, serum creatinine and SDMA levels, venous pH, urinary density, and UPC for each of the five treated dogs are shown in Table 2.

Maintenance of most values was observed, as shown in Table 2. The number of leukocytes decreased in all dogs. In the dog 5, the serum creatinine level fell from 3.5 mg/dL to 2.4 mg/dL from day 0 to day 153; the levels in dogs 3 and 4 remained stable.

Cylinduria was observed in one dog (dog 4) at the beginning of the study. This patient had 4–6 hyaline cylinders and 1–2 granular cylinders per field. During the treatment, a reduction in the quantification of cylinders was observed, and from day 63, no cylinders were observed.

Considering the five treated animals, there was no statistical difference in blood cell counts; creatinine, urea, sodium, potassium, calcium, and phosphorus levels; or urinary density over the study period ($p>0.05$) (Table 3).

The details of each of the treated animals and the information reported by the owners are shown in Table 4. Dog 1 gained 40 g of body weight, stopped vomiting, and had increased activity. Dog 3 had decreased urinary frequency and increased activity. Dog 4 gained 600 g of body weight. Dog 5 gained 100 g of body weight and had increased activity.

DISCUSSION

The main objective of the present study was to evaluate the safety of the administration of aASCs in dogs with CKD, since this has not yet been demonstrated in this species and in these specific clinical conditions. It was observed that the intravenous infusion of aASCs did not present any adverse effects during the immediate period after any of the three infusions or during the follow-up period of four months after it.

Table 2. Clinical (body weight and systolic blood pressure) and laboratory examination (blood erythrocytes and leukocytes, creatinine, SDMA, venous pH, urinary specific gravity, and UPC) of dogs with chronic kidney disease treated with adipose tissue-derived allogeneic mesenchymal stromal cells

		Evaluation Days				
		0	21	42	63	153
Dog 1	Body weight	3.8	3.8	3.8	3.7	3.8
	SBP	U	170	160	131	115
	Erythrocytes	8.68	8.61	8.09	8.18	7.34
	Leukocytes	8.3	8.1	7.4	7.7	6.2
	Creatinine	2.1	2.1	2.2	2.3	2.6
	SDMA	35	35	37	44	39
	Venous pH	7.26	7.3	7.3	7.29	7.29
	Urinary specific gravity	1.018	1.018	1.018	1.016	1.015
	UPC	0.10	0.10	0.12	0.13	NR
Dog 2	Body weight	9.3	9.2	9.2	9.2	9.2
	SBP	250	U	180	U	U
	Erythrocytes	7.05	7.38	7.62	6.80	7.1
	Leukocytes	8.1	7.2	8.3	7.5	6.6
	Creatinine	1.2	1.4	1.5	1.6	1.6
	SDMA	17	17	20	18	27
	Venous pH	7.35	7.35	7.33	7.34	7.3
	Urinary specific gravity	1.015	1.018	1.016	1.020	1.016
	UPC	U	U	0.40	U	0.50
Dog 3	Body weight	20.5	20.9	20.5	20.4	20.4
	SBP	125	U	180	U	U
	Erythrocytes	8.46	8.29	8.50	7.87	8.07
	Leukocytes	8.1	6.8	6.3	7.6	6.6
	Creatinine	2.1	1.8	1.8	1.7	2.0
	SDMA	44	19	21	20	15
	Venous pH	7.43	7.38	7.38	7.34	7.3
	Urinary specific gravity	1.006	1.015	1.016	1.014	1.016
	UPC	U	0.09	0.71	0.13	0.51
Dog 4	Body weight	6.0	6.3	6.2	6.6	6.6
	SBP	120	U	116	U	U
	Erythrocytes	8.82	7.39	7.53	7.97	6.95
	Leukocytes	9.2	8.8	9.5	10.8	8.1
	Creatinine	1.8	1.9	1.8	2.0	1.7
	SDMA	20	15	16	17	22
	Venous pH	7.38	7.38	7.38	7.39	7.4
	Urinary specific gravity	1.030	1.028	1.026	1.022	1.030
	UPC	U	U	0.62	U	0.49
Dog 5	Body weight	7.8	7.9	7.9	7.9	7.9
	SBP	210	130	130	U	U
	Erythrocytes	5.12	5.7	5.36	4.9	5.01
	Leukocytes	8.8	7.5	7.8	7.1	6.5
	Creatinine	3.5	2.1	2.7	3.1	2.4
	SDMA	19	27	20	28	33
	Venous pH	7.36	7.31	7.35	7.36	7.41
	Urinary specific gravity	1.020	1.018	1.020	1.020	1.018
	UPC	U	U	1.1	0.72	0.87

Body weight, kg; SBP, systolic blood pressure, mmHg; erythrocytes, millions/mm^3; leukocytes, thousand/mm^3; creatinine, mg/dL; SDMA, symmetrical dimethylarginine, mg/dL; UPC, urine protein to creatinine ratio; U, unrealized.

Table 3. Summary (median, standard deviation) of laboratory data of dogs with chronic kidney disease treated with adipose tissue-derived allogeneic mesenchymal stromal cells

Parameters	Day 0	Day 21	Day 42	Day 63	Day 153
Blood Count					
Erythrocytes (millions/mm^3)	7.6±1.6	7.5±1.1	7.4±1.22	7.1±1.4	6.9±1.0
Hematocrit (%)	51±9.35	51±8.5	50.8±9.7	48.8±10.2	47±8.2
Leukocytes (thousand/mm^3)	8.5±0.5	7.7±0.8	7.9±1.2	8.1±1.5	6.8±0.7
Platelets (thousand/mm^3)	356.4±71	419±105.6	283.2±84.5	339±61.6	338.4±82.7
Total protein (g/dL)	7.2±0.7	7.3±0.8	7.6±0.7	7.3±0.72	7.3±0.7
Seric Biochemistry					
Urea (mg/dL)	84.4±30.4	82.4±31.5	90.4±24.4	95±27.9	88.4±26.7
Creatinine (mg/dL)	2.1±0.8	1.9±0.3	1.9±0.3	2.1±0.6	2.1±0.4
SDMA (mcg/dL)	27.6±11.5	22.6±8.29	22.8±8.17	25.4±11.3	27.2±8.35
Blood Gas Analysis					
pH	7.4±0.06	7.3±0.05	7.3±0.03	7.4±0.06	7.4±0.05
pCO_2 (mm/Hg)	37±5.2	36.5±6.2	34.7±3.7	33±5.7	34.1±7.3
HCO_3 (mEq/L)	20.2±1.05	19.3±0.9	19±1.2	19±2.8	19.8±2.4
BE	-4.6±2.3	-6.2±1.8	-6.0±1.1	-6.1±3.42	-5.6±2.5
Sodium (mEq/L)	145±2.24	144.6±2.2	145.2±1.3	144.4±1.52	147±1.7
Potassium (mEq/L)	4.6±0.53	4.5±0.4	4.9±0.44	4.6±0.5	4.6±0.3
Ionic Calcium (mEq/L)	1.4±0.11	1.4±0.05	1.4±0.04	1.3±0.04	1.4±0.04
Phosphorus (mg/dL)	3.2±0.4	3.5±0.6	3.7±0.4	5.2±3.1	3.5±0.8
Urinalysis					
Urinary specific gravity	1.02±0.01	1.019±0.005	1.019±0.004	1.018±0.003	1.019±0.006
pH	5.6±0.9	5.8±0.4	5.8±0.4	5.8±0.4	5.9±1.1
Glucose	Negative	Negative	Negative	Negative	Negative
Cylinders	Hyaline(1dog)	Hyaline(1dog)	Hyaline(1dog)	Negative	Negative
UPC	0.1-1.3 (2 dogs)	0.7 (3 dogs)	0.8 ± 0.4	0.8 (3 dogs)	0.5 (3 dogs)

UPC, urine protein to creatinine ratio

Table 4. Clinical signs and behavior (according to owners) of dogs with chronic kidney disease before and after treatment with adipose tissue-derived allogeneic mesenchymal stromal cells

Animal	Clinical signs before treatment	Clinical signs after treatment	Behavior before treatment	Behavior after treatment
1	Sporadic vomiting, pasty feces, hypertension	No vomiting, normal feces, normotension, +40g BW	Lethargy, hyporexia	↑ Activity, normorexia
2	Unchanged	Unchanged	Unchanged	Unchanged
3	Polydipsia, nocturia	↓ Water intake, ↓ Urinary frequency	Unchanged	↑ Activity
4	Unchanged	+600g BW	Unchanged	Unchanged
5	Unchanged	+100g BW	Lethargy	↑ Activity

BW, body weight; g, grams; ↑, increased; ↓, decreased; +, gained.

It was decided to use allogeneic cells, as it is understood that in the case of patients with CKD, performing anesthetic and surgical procedures to collect adipose tissue could worsen the delicate clinical condition. We chose to prepare MSCs for administration by intravenous infusion as it is a simpler procedure and has a lower risk of complications in patients with CKD.

The five animals investigated in the present study were followed up for a period of 120 days after treatment, which allowed us to assess the safety of aASC treatment. However, for the evaluation of the therapeutic effects in dogs with CKD, a larger sample would be necessary. Moreover, even though routine clinical markers were included, these were of limited value to prove the beneficial action of the aASCs. Non-routine tests would be necessary, such as renal biopsy with histopathological evaluation and interleukin evaluation, or it would be necessary to evaluate a larger number of patients.

Thus, the statistical evaluation of clinical findings and biomarkers of renal function and injury did not show any difference between the different assessments, but it is believed that some of the identified observations have clinical relevance and therefore deserve to be emphasized and discussed to serve as inspiration for future studies.

Initially, dogs that showed clinical signs of CKD at the beginning of treatment showed improved symptoms. Dogs with CKD under conventional treatment may show clinical improvement but supporting medication must often be maintained (Roudebush *et al*., 2010). Therefore, it is suggested that the clinical improvement in the animals investigated in the present study might be due to the participation of ASCs in controlling the disease and improving their quality of life because they did not use any other medication during this study.

In this study, there was a gain in body weight in dogs treated with ASCs, showing an important beneficial result of treatment with cell therapy, since the loss of body weight usually occurs with the progression of the disease. Weight loss can be caused by numerous factors that result from inflammation, such as increased protein catabolism and increased excretion of bicarbonate, which leads to metabolic acidosis that stimulates muscle cell breakdown and loss of lean muscle. In addition, the cytokines, IL-6 and TNF-α, also act by compromising appetite, leading to a spontaneous reduction in food intake (Oliveira *et al*., 2010). Loss of body weight is a risk factor that increases the rates of morbidity and mortality in people and dogs with chronic diseases (Freeman, 2012; Ineson, Freeman and Rush, 2019). In this study, the anti-inflammatory

action of MSCs may have been the mechanism that underlay the improvement and maintenance of body weight in dogs with CKD; further studies are needed to better understand the mechanisms involved as this may influence the survival rate.

In addition, the dogs were more active after infusion of ASCs according to their owners. This leads us to think that because they were elderly animals and may have other comorbidities, such as joint disease, they can benefit from the treatment performed. MSCs may have migrated to other inflammatory foci, such as the joints, resulting in improved disposition and activity; however, in addition to the possible control of inflammatory foci in the joints, we hypothesize that the kidney is also beneficial. This factor is known as homing, which is the ability of stem cells to find their destination in a target organ through the bloodstream, where it directs the migration of stem cells through different signaling pathways, mediated by cytokines or receptors for growth factors released on the surface of stem cells (Tao *et al*., 2018).

Hematological tests did not show any significant changes. The number of leukocytes decreased during the study period, which may suggest further evidence of treatment safety in this study, due to the absence of any type of adverse inflammatory response resulting from allogeneic cell treatments.

As for biomarkers, such as creatinine used to assess renal function, it was observed that there was a decrease in one of the treated dogs and in two dogs, the level remained stable. This may indicate a beneficial treatment effect on the control or even improvement of the clinical picture, which would require a new study with a larger number of animals for confirmation. A decrease in serum creatinine levels in patients with CKD stages II and III was reported in cats, at 7 and 60 days after the infusion of allogeneic amniotic membrane-derived MSCs (Vidane *et al*., 2017, showing a renoprotective effect of cell therapy in CKD.

Cylinduria was observed in only one of the investigated dogs, with complete resolution at the end of the treatment. These results suggest an improvement in the condition of the tubular epithelium. The effects of ASCs on the formation of cylinders in kidney disease have not been previously demonstrated in domestic animals; however, ASCs attenuate markers of kidney injury and oxidative damage, protect the tubular epithelium from reperfusion injury, inhibit apoptosis in the injured region, and increase the proliferation of surviving cells in a study carried out in rats (Zhuo *et al*., 2013).

Other limitations of this study would include the evaluation of UPC, which was not performed at all the stages for laboratory reasons, since this test was only performed if proteinuria was present. UPC would help us to establish a prognosis because persistent proteinuria is associated with the risk of uremic morbidity and mortality, and we suggest that it should be included consistently in future studies. SBP was not measured at all stages, as some patients were very stressed at the time of the clinical examination. However, two patients showed a reduction in SBP during treatment, and for these, we have two hypotheses: that they had already accustomed themselves to the outpatient clinic, staff, and treatment, or that it may have been another beneficial ASC effect. Additionally, future studies should include the evaluation of the glomerular filtration rate and analysis of urinary cytokines. The low number of animals surprised us because we believed that we would be able to recruit a larger number of cases to better answer the study questions; however, the adherence of owners and veterinarians was low, showing what can be inherent in a study involving routine cases.

CONCLUSION

In conclusion, this study showed that cell therapy with intravenous administration of aASCs in dogs with CKD was safe. Although some benefits were observed in the present study, this must be further investigated in a larger number of cases.

REFERENCES

CHEW, D.J.; DIBARTOLA, S.P.; SCHENCK, P.A. *Urologia e nefrologia do cão e do gato*. 2.ed. Rio de Janeiro: Elsevier, 2011.

COWGILL, L.D.; POLZIN, D.J.; ELLIOTT, J. *et al*. Is progressive chronic kidney disease a slow acute kidney injury? *Vet. Clin. North Am. Small Anim. Pract.*, v.46, p.995-1013, 2016.

FALCÃO, M.S.A.; BRUNEL, H.S.S.; PEIXER, M.A.S. *et al*. Effect of allogeneic mesenchymal stem cells (MSCs) on corneal wound healing in dogs. *J. Tradit. Complementary Med.*, v.10, p.440-445, 2020.

FREEMAN, L.M. Cachexia and sarcopenia: emerging syndromes of importance in dogs and cats. *J. Vet. Intern. Med.*, v.26, p.3-17, 2012.

INESON, D.L.; FREEMAN, L.M.; RUSH, J.E. Clinical and laboratory findings and survival time associated with cardiac cachexia in dogs with congestive heart failure. *J. Vet. Intern. Med.*, v.33, p.1902-1908, 2019.

IRIS staging of CKD modified 2019. International Renal Interest Society (IRIS). Available in: http://www.iriskidney.com/pdf/IRIS_Staging_of_CKD_modified_2019.pdf. Accessed in: 2 Oct. 2019.

KOGIKA, M.M.; LUSTOZA, M.D.; HAGIWARA, M.K. *et al*. Evaluation of oxidative stress in the anemia of dogs with chronic kidney disease. *Vet. Clin. Pathol.*, v.44, p.70-78, 2015.

LE BLANC, K.; TAMMIK, C.; ROSENDAHL, K. *et al*. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp. Hematol.*, v.31, p.890-896, 2003.

LUND, E.M.; ARMSTRONG, P.J.; KIRK, C.A.; KOLAR, L.M.; KLAUSNER, J.S. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. *J. Am. Vet. Med. Assoc.*, v.214, p.1336-1341, 1999.

OLIVEIRA, C.M.C.; KUBRUSLY, M.; MOTA, R.S.; SILVA, C.A.B.; OLIVEIRA, V.N. Malnutrition in chronic kidney failure: what is the best diagnostic method to assess? *Braz. J. Nephrol.*, v.32, p.57-70, 2010.

QUIMBY, Jessica M. *et al*. Evaluation of intrarenal mesenchymal stem cell injection for treatment of chronic kidney disease in cats: a pilot study. *Journal of feline medicine and surgery*, v. 13, n. 6, p. 418-426, 2011.

QUIMBY, Jessica M. *et al.* Safety and efficacy of intravenous infusion of allogeneic cryopreserved mesenchymal stem cells for treatment of chronic kidney disease in cats: results of three sequential pilot studies. *Stem cell research & therapy*, v. 4, n. 2, p. 1-12, 2013.

PELANDER, L.; LJUNGVALL, I.; EGENVALL, A. *et al*. Incidence of and mortality from kidney disease in over 600,000 insured Swedish dogs. *Vet. Rec.*, v.176, p.656, 2015.

POLZIN, D.J. Chronic kidney disease in small animals. *Vet. Clin. North Am. Small Anim. Pract.*, v.41, p.15-30, 2011.

ROUDEBUSH, P.; POLZIN, D.J.; ADAMS, L.G.; TOWELL, T.L.; FORRESTER, S.D. *et al*. An evidence-based review of therapies for canine chronic kidney disease. *J. Small Anim. Pract.*, v.51, p.244-252, 2010.

SUTTON, T.A.; FISHER, C.J.; MOLITORIS, B.A. Microvascular endothelial injury and dysfunction during ischemic acute renal failure. *Kidney Int.*, v.62, p.1539-1549, 2002.

TAO, Z.; TAN, S.; CHEN, W.; CHEN, X. Stem cell homing: a potential therapeutic strategy unproven for treatment of myocardial injury. *J. Cardiovas. Transl. Res.*, v.11, p.403-411, 2018.

THOMSON, A.L.; BERENT, A.C.; WEISSE, C.; LANGSTON, C.E. Intra-arterial renal infusion of autologous mesenchymal stem cells for treatment of chronic kidney disease in cats: phase I clinical trial. *J. Vet. Intern. Med.*, v.33, p.1353-1361, 2019.

VIDANE, A.S.; PINHEIRO, A.O.; CASALS, J.B. *et al*. Transplantation of amniotic membrane-derived multipotent cells ameliorates and delays the progression of chronic kidney disease in cats. *Reprod. Domest. Anim.*, v.52, Suppl. 2, p.316-326, 2017.

VILLANUEVA, S.; GONZÁLEZ, F.; LORCA, E. *et al*. Adipose tissue-derived mesenchymal stromal cells for treating chronic kidney disease: a pilot study assessing safety and clinical feasibility. *Kidney Res. Clin. Pract.*, v.38, p.176-185, 2019.

ZHUO, W.; LIAO, L.; FU, Y.; XU, T. *et al*. Efficiency of endovenous versus arterial administration of mesenchymal stem cells for ischemia-reperfusion–induced renal dysfunction in rats. *Transplant. Proc*., v.45, p.503-510, 2013.