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Canine adipose tissue-derived mesenchymal stem cell therapy in a dog with renal Fanconi syndrome

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Abstract: Renal Fanconi syndrome (RFS) affects the proximal tubular resorption in the nephrons. This causes excessive loss of key solutes through the urine. In a canine patient, we successfully managed the renal tubular acidosis and proteinuria caused by RFS via transplantation of canine adipose tissue-derived mesenchymal stem cells (cAT-MSCs). cAT-MSCs were administered ten times at intervals of 2–4 weeks. The post-therapy check-up revealed that the cAT-MSC treatment improved the renal tubular acidosis and proteinuria. Hence, a cAT-MSC transplant may be considered as an adjuvant therapy in veterinary medicine to initiate and maintain relief of RFS-induced acidosis and proteinuria.

Keywords: glucosuria; proteinuria; renal tubular acidosis

Renal Fanconi syndrome (RFS) is a disorder of the proximal renal tubules in the nephrons that leads to the improper reabsorption of electrolytes and nutrients as they, instead, spill into the urine (Yearley et al. 2004). An effective treatment to reverse and potentially cure the syndrome has not been reported to date (Karatzas et al. 2017). Disease management depends on the severity of the impaired resorption; hence, a personalised therapy is essential. Supplementation of the potassium, nutritional management for kidney diseases, and support to re-establish the body's acid-base balance are the common treatments (Gonto 2003).

Mesenchymal stem/stromal cells (MSCs) are undifferentiated adult stem cells mostly isolated from the adipose tissue, the umbilical cord or placenta, and the bone marrow. They can be differentiated into adipocytes, chondroblasts, and osteoblasts (Soleymaninejadian et al. 2012). Reports have ascertained that the renoprotective effects of MSCs can occur because of paracrine signal release, stimulation of the local endogenous repair, and fusion or engraftment into host tissues (Lange et al. 2005).

However, in veterinary medicine, use of MSCs for RFS treatment in dogs has not been reported upon till date to the best of our knowledge. This case report describes the first successful management of renal tubular acidosis and proteinuria in RFS using canine adipose tissue-derived mesenchymal stem cells (cAT-MSCs).

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https://doi.org/10.17221/213/2020-VETMED

Case description

A 7-year-old, 3.0 kg, spayed female Poodle previously diagnosed with exocrine pancreatic insufficiency (EPI) was presented to the Veterinary Medical Teaching Hospital of Seoul National University with polyuria and polydipsia. Following the diagnosis of EPI, the patient had been treated with vitamin B12 (0.5 mg/kg subcutaneously once per 2 weeks) and pancreatin (Norzyme cap. 10000; Pharmbio Korea, Seoul, Republic of Korea).

The serum biochemistry revealed hypophosphatemia [phosphorus (IP), 0.71 mmol/l, reference: 0.74–1.77 mmol/l, hypoglycaemia (glucose, 2.83 mmol/l, reference: 3.33–6.66 mmol/l) and low potassium (K^+) levels (K^+, 2.93 mmol/l, reference: 3.9–5.1 mmol/l). The dog was not azotemic, and the liver enzymes were not elevated during this period. Urine samples collected via cystocentesis repeatedly tested positive for the presence of protein (1+), glucose (3+), and ketone (trace) on a dipstick analysis, with a urine-specific gravity of 1.007 (reference: 1.030–1.050). A urine smear did not reveal bacterial presence, while the urine protein-to-creatinine ratio measurements and venous blood gas analysis revealed proteinuria (3.00) and metabolic acidosis (pH 7.21, reference: 7.31–7.46; bicarbonate, 12.4 mmol/l, reference 21–23 mmol/l), respectively. Considering the above results, the dog was suspected to have Fanconi syndrome. The Fanconi syndrome screening test (IDEXX, Seoul, Republic of Korea) results revealed severe aminoaciduria, glucosuria, and lactic aciduria, consistent with the Fanconi syndrome. To exclude the differential diagnoses, serological tests for toxic heavy metals, such as mercury, zinc, copper and lead (Neodin BioVet Laboratory, Seoul, Republic of Korea), were performed, and these revealed no abnormalities. The anamnesis revealed that the dog was fed commercial food (intestinal low fat; Royal Canin, Aimargues, France) and had never consumed other foods or treats like Chinese chicken jerky (Yabuki et al. 2017), which is well-known to cause of RFS in dogs. After being diagnosed with RFS, the dog underwent treatment based on the Gonto protocol (Gonto 2003). The dog was treated with sodium bicarbonate (Tasna tab; Nexpharm Korea, Seoul, Republic of Korea) for renal tubular acidosis and proteinuria, and treatment with enalapril (Enalapril tab; Dongkwang, Seoul, Republic of Korea), spironolactone (Aldactone film-coated tab; Pfizer Inc, New York, USA), and telmisartan (Semintra oral solution; Boehringer Ingelheim, Ingelheim, Germany) was started.

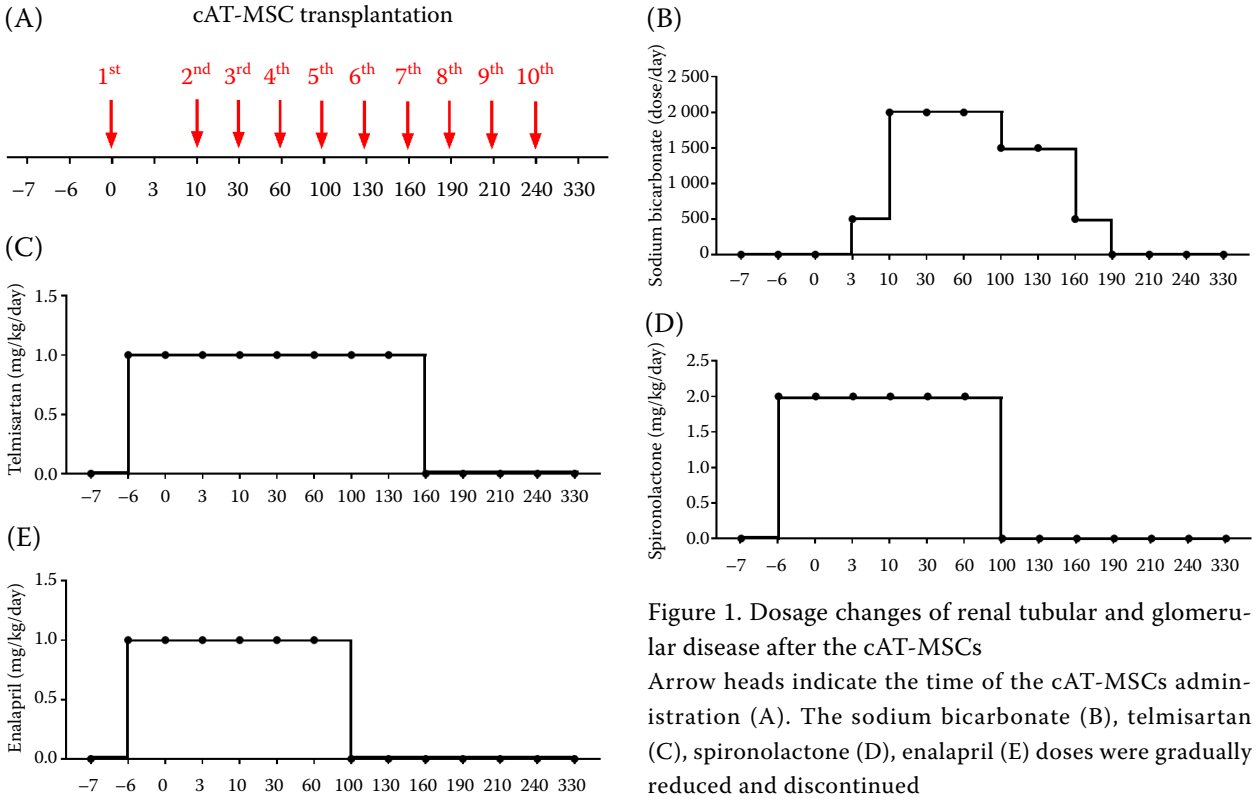

Figure 1. Dosage changes of renal tubular and glomerular disease after the cAT-MSCs

Arrow heads indicate the time of the cAT-MSCs administration (A). The sodium bicarbonate (B), telmisartan (C), spironolactone (D), enalapril (E) doses were gradually reduced and discontinued

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After that, to aid the renal regeneration, an allogeneic transplantation of cAT-MSCs was performed. The consent of the patient's owner was obtained before applying the stem cells as a treatment. After the initial administration of cAT-MSCs, no cAT-MSC-related side effects were observed, and the patient's vitality improved. The stem cells were applied in consideration of the dose previously applied to dogs for treatment (Perez-Merino et al. 2015). This patient received 2×10^6 cells/kg cAT-MSCs intravenously at intervals of 2–6 weeks over a period of 9 months, ten times in total (Figure 1A). Three to four passages of fresh stem cells applied as therapeutics to patients were collected from the adipose tissue from healthy dogs, and the stem cell extraction method was carried out by a previously established method (Yang et al. 2018). The cAT-MSCs positively expressed CD29, CD44, and CD90 and did not express the surface markers CD34 and CD45 by flow cytometry. In addition, it was confirmed

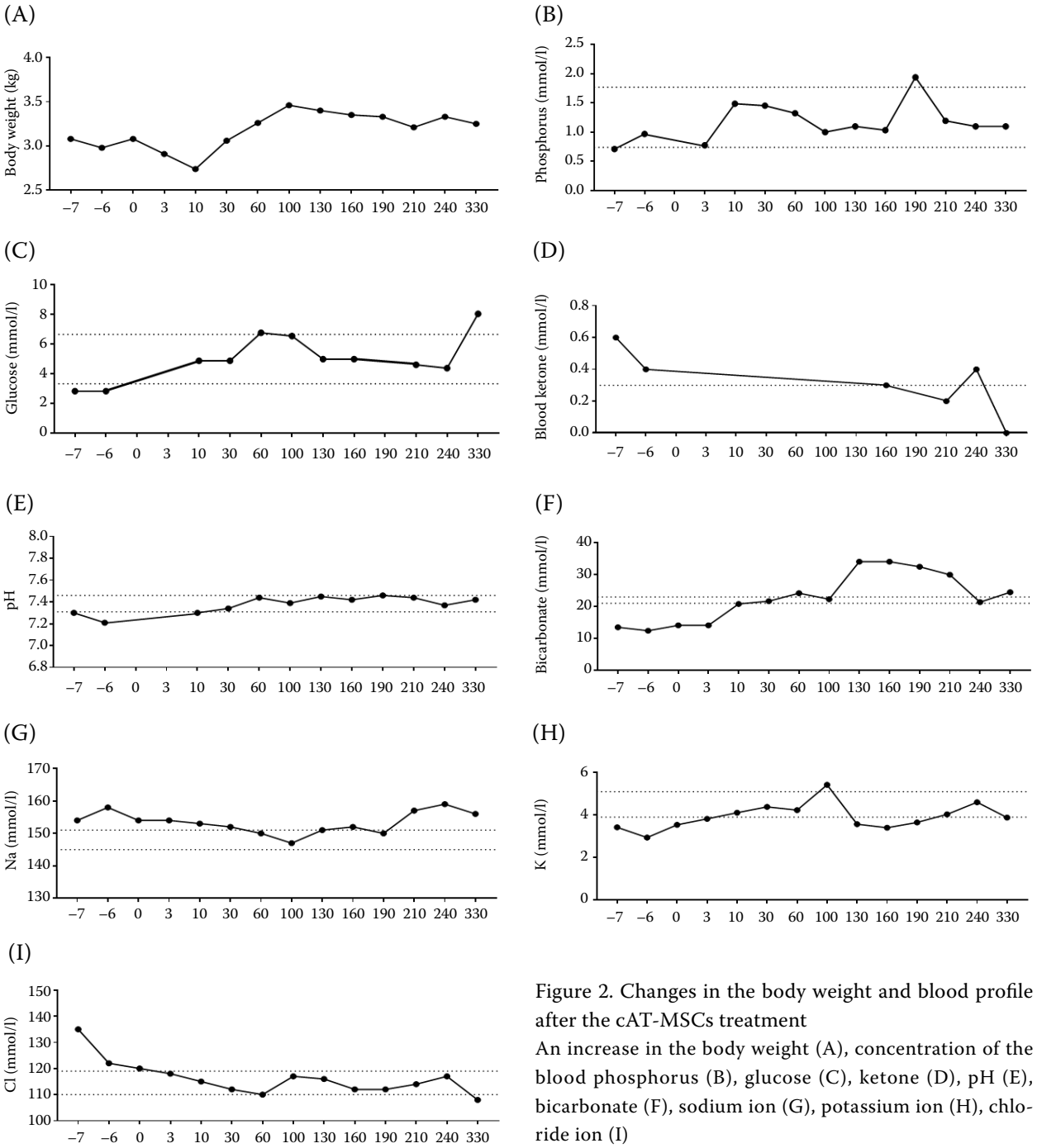

Figure 2. Changes in the body weight and blood profile after the cAT-MSCs treatment

An increase in the body weight (A), concentration of the blood phosphorus (B), glucose (C), ketone (D), pH (E), bicarbonate (F), sodium ion (G), potassium ion (H), chloride ion (I)

https://doi.org/10.17221/213/2020-VETMED

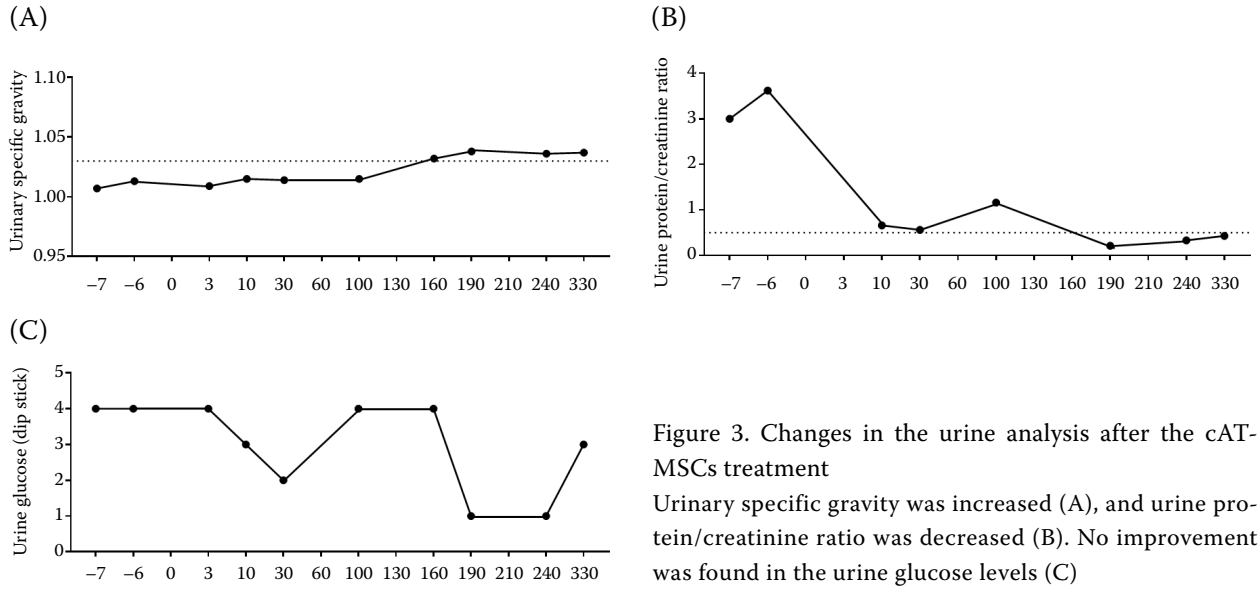

Figure 3. Changes in the urine analysis after the cAT-MSCs treatment

Urinary specific gravity was increased (A), and urine protein/creatinine ratio was decreased (B). No improvement was found in the urine glucose levels (C)

that the stem cells have the ability to differentiate into adipocytes, osteocytes, and chondrocytes. These data are not shown. During this period, repeated laboratory evaluations revealed progressive lowering of the proteinuria, renal tubular acidosis, electrolyte imbalance, hypoglycaemia, and hypophosphatemia.

The dosages of the sodium bicarbonate and proteinuria-related drugs were gradually tapered during the initial 6 months (Figure 1) and discontinued during the final 3 months. Since termination of the cAT-MSC treatment, the management of the renal tubular acidosis and proteinuria required no additional treatment. Furthermore, the body weight, and blood glucose and blood phosphorus levels increased postoperatively; however, the dog's electrolyte imbalance was not identified (Figure 2). A urine analysis showed lowering in the proteinuria (Figure 3), which remained manageable for 3 months after the final cAT-MSC treatment.

DISCUSSION AND CONCLUSIONS

The diagnosis of idiopathic RFS was made according to the following criteria: (1) excessive urinary excretion of amino acids, (2) reduced serum phosphate in an unrestricted diet, and (3) evidence of type 2 (proximal) renal tubular acidosis, including metabolic acidosis (hyperchloremic) (Karatzas et al. 2017). RFS can be temporary in dogs due to early reversible causes, but, in most affected dogs, the disease is chronic and persistent (Hostutler et al. 2004). As described in the literature on the long-term prognosis of affected dogs, many either died or were euthanised shortly after the diagnosis (Yearley et al. 2004). The cAT-MSCs treatment provides a method for prolonging the lives of canines who would otherwise be euthanised.

Since the underlying defects responsible for the development of the Fanconi syndrome in dogs have not been fully characterised, treatment recommendations focus on using supplements to minimise the systemic effects of the chronic large-scale loss of key solutes (Ali and Tariq 2016). The recommended treatments in the human and veterinary literature have generally focused on controlling the chronic acidosis and electrolyte abnormalities, with a particular emphasis on potassium and phosphate supplementation (Wilson and Yendt 1963).

In patients with Fanconi syndrome, precautions are necessary to prevent side effects during the acidosis treatment with bicarbonates. Although high-dose bicarbonate supplementation may be necessary due to the persistent tubular bicarbonate losses, some aspects of the supplementation have been anecdotally reported to cause mild neurological dysfunctions (Hu et al. 2019). For example, dogs receiving high-dose alkaline supplements twice daily may develop transient mild ataxia or dementia immediately after administration (Yearley et al. 2004).

Some previous studies reported that the average survival time was longer when RFS was diagnosed early, as 50% of the dogs affected in a previous study died within 90 days of the diagnosis. Similarly, 12 of 21

https://doi.org/10.17221/213/2020-VETMED

(57%) dogs described in a previous report survived < 5 months after diagnosis (Easley and Breitschwerdt 1976). Most dogs with Fanconi syndrome did not have azotaemia at the time of diagnosis, but the likelihood of kidney failure was a clinically important concern. Although not all dogs with RFS develop kidney failure, it is a common finding and has been reported in several dogs (Norden et al. 2001). Chronic proteinuria may contribute to kidney failure in dogs with RFS (Nakhoul and Batuman 2011).

The mechanism by which stem cells treat renal tubular and glomerular damage has not yet been fully elucidated upon. A previous study demonstrated that the renoprotective activity of stem cells (both in the acute and chronic renal disease models) is due to the stem cell secretion of cytokines and other molecules that inhibit inflammation and fibrosis and promote endogenous repair processes, including angiogenesis (de Almeida et al. 2013). These renoprotective properties have an attractive therapeutic potential for treating renal tubular and glomerular diseases (Rabelink and van Kooten 2006).

This case report described the clinical application of cAT-MSCs for renal tubular acidosis and proteinuria in a canine RFS patient. The initial treatment after the RFS diagnosis consisted of a high-dose bicarbonate supplementation to control the metabolic acidosis and electrolyte abnormalities. Also, enalapril, spironolactone, and telmisartan were administered orally to reduce the proteinuria. After administration of the cAT-MSCs, the need for the sodium bicarbonate and proteinuria-related drugs gradually decreased, and 180 days after stem cell administration, the patient was well managed, without acidosis and proteinuria, despite the absence of these drugs. After the end of the cAT-MSC treatment, the metabolic acidosis and proteinuria were well controlled for three months without any related drugs. In this case, there is a limitation in that there is no long-term follow-up of more than one year after the end of stem cell treatment, so it is not possible to know how long the treatment effect will last. In addition, it is known that there are various methods of injecting stem cells, but in this patient, the stem cells were injected intravenously to evaluate the therapeutic effect. Evaluating the effectiveness of the treatment with other injection methods, such as the direct injection into the organ with ultrasound guidance, would be beneficial for future studies (Beerts et al. 2021). In this case, only stem cells were injected alone without any other drug, but in order to increase the efficiency of the stem cell therapy, it would be good to consider applying such a vehicle to patients receiving stem cells. In addition, it cannot be excluded that autoimmunity may cause damage to the glomeruli and tubules in a patient with a previously diagnosed EPI (Evans et al. 2020). In the case of stem cells, there is a report where regulatory T-cells are activated to stabilise the abnormal immunity and restore kidney damage (Sharma and Kinsey 2018). In this case presentation, studies on the mechanisms related to immune cell regulation after stem cell administration in RFS have not yet been conducted, but future studies on this should be conducted.

To the best of our knowledge, this is the first report describing the application of cAT-MSCs for treating a case of canine RFS and its successful management, leading to clinical remission. Although additional studies are needed to further investigate the clinical applications of cAT-MSCs in renal tubular diseases, a cAT-MSC transplantation could be proposed as a novel treatment method for renal tubular acidosis and proteinuria of RFS in veterinary medicine.

Conflict of interest

The authors declare no conflict of interest.

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