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Combination of immunosuppressive drugs and allogeneic stem cell treatment in a dog with suspected nephrotic syndrome

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Abstract: The case study aims to describe the nephrotic syndrome (NS) in a castrated 3-year-old male Cocker Spaniel dog. The patient arrived at the hospital with a loss of appetite and weakness. Skin oedema with ascites was observed along with hypoalbuminaemia, hypoproteinaemia, hyperlipidaemia, hypercholesterolaemia, and proteinuria (urine protein to creatinine ratio = 22.4). Based on these findings, the patient was diagnosed with NS, although a renal biopsy was not conducted. Prednisolone (1 mg/kg, p.o. q12 h) and mycophenolate mofetil (10 mg/kg, p.o. q12 h) were prescribed as the immunosuppressive drugs, and previously cryopreserved allogeneic adipose tissue-derived mesenchymal stem cells (2×10^7 cells/kg) were injected intravenously. After several weeks of treatment, the patient recovered from NS. This is the first case report on immunosuppressive drugs and allogeneic mesenchymal stem cells being used to treat a dog with NS.

Keywords: dog; glomerular disease; immunosuppressive drug; mesenchymal stem cell; nephrotic syndrome

Nephrotic syndrome (NS) is characterised by a series of symptoms caused by glomerular damage. Moreover, proteinuria, hypoalbuminaemia, hyperlipidaemia, and significant oedema caused by fluid accumulation in the interstitial space are all symptoms of NS (Klosterman and Pressler 2011; Klosterman et al. 2011). Proteinuria develops as a result of glomerular damage, which decreases the selective permeability (Klosterman et al. 2011). When the urinary protein loss exceeds the rate of the hepatic albumin synthesis, hypoalbuminaemia occurs. Hypoalbuminaemia can result in an increase in the hepatic biosynthesis that is not specific, resulting in hypercholesterolaemia (Kronenberg 2005). This condition is uncommon in dogs and has a short median survival time (Klosterman and Pressler 2011; Klosterman et al. 2011).

The treatments for NS are the management of the complications and related therapies recommended in dogs with glomerular disease (Klosterman and Pressler 2011). Generally, after a renal biopsy and histopathological confirmation, clinicians should prescribe immunosuppressive drugs to dogs with glomerular disease (Segev et al. 2013). However, if the hypoalbuminaemia is severe, an aggressive immunosuppressive treatment can be suggested to patients with NS (Pressler et al. 2013).

Although mesenchymal stem cells are used to control several diseases in veterinary medicine (Quimby et al. 2013; Perez-Merino et al. 2015; Lee et al. 2017; Dias et al. 2019), their ability to treat dogs with NS is unknown. However, several studies reported that mesenchymal stem cells inhibit inflammation and initiate tissue regeneration

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(Kode et al. 2009; Teng et al. 2015; Pittenger et al. 2019). In addition, mesenchymal stem cells are being studied regarding kidney disorders in human medicine due to their therapeutic potential (Peired et al. 2016; Bochon et al. 2019).

Based on the clinical and laboratory findings, the dog in this study was diagnosed with NS. The dog was administered immunosuppressive drugs and intravenous injections of mesenchymal stem cells, which then successfully recovered.

To the best of our knowledge, this is the first report of a dog with NS treated with immunosuppressive medications and allogeneic adipose tissue-derived mesenchymal stem cells.

Case report

A previously healthy three-year-old castrated male Cocker Spaniel dog was presented with a 1-week history of anorexia and decreased activity level. On physical examination, the patient was observed to be dehydrated with a pink and dry mucous membrane. The abdomen was dilated, and the systolic blood pressure of the patient was 130 mmHg. A complete blood count, serum biochemistry, and urinalysis were administered and then evaluated. Hyperlipidaemia [1.65 g/l; reference index (RI): 0.3–1.33 g/l], hypercholesterolemia (19.72 mmol/l; RI: 6.17–17.33 mmol/l), hypoalbuminaemia (13 g/l; RI: 24–35 g/l), and hypoproteinaemia (45 g/l; RI: 50–72 g/l) were found. All of these exceptions were observed to be normal upon the blood examination. The urinalysis revealed proteinuria (urine protein to creatinine ratio = 22.4) with an adequate urine concentration and no evidence of urinary tract infection. Furthermore, a small volume of ascites was discovered via abdominal ultrasonography. Based on these findings, the dog was diagnosed with glomerular disease-related NS.

Due to the hypoalbuminaemia and the associated complications, benazepril (0.5 mg/kg, p.o. q12 h) and clopidogrel (1 mg/kg, p.o. q12 h) were administered. Despite these treatments, the dog's condition continued to deteriorate after a day. The ascites became large enough to warrant an abdominocentesis and were diagnosed as transudate.

Although the histological diagnosis of immune-mediated glomerular disease was not confirmed, it was decided to use an immunosuppressive drug based on the clinical presentation and laboratory findings. As immunosuppressants, prednisolone (1 mg/kg, p.o. q12 h) and mycophenolate mofetil (10 mg/kg, p.o. q12 h) were administered. Omeprazole (1 mg/kg, p.o. q12 h) was also administered to reduce the gastrointestinal damage.

Despite these treatments, the dog's condition and hypoalbuminaemia (11 g/l) worsened. Thus, 10 g of human serum albumin was infused intravenously with normal saline for 12 hours. After adjustment of the human serum albumin, the plasma albumin of the patient was increased to 22 g/l. However, after 24 h, the albumin of the patient was decreased to 17 g/dl. Therefore, we suggested cryopreserved allogeneic mesenchymal stem cell treatment as a clinical trial after consulting with the owner.

Mesenchymal stem cells (2×10^7 cells/kg) with normal saline were injected into the patient intravenously for 15 minutes. The drug medication was maintained.

After the stem cell infusion, the plasma albumin concentration was increased to 20 g/l. Over the next two days, the plasma albumin concentration was 24 g/l, and the dog's condition improved sig-

Table 1. Urinalysis of the patient

	Day 0	Day 2	Day 3	Day 18	Day 33
Colour	yellowish green	yellow	yellow	yellow	–
Turbidity	cloudy +++	cloudy +++	cloudy +	cloudy +	–
Stick	protein +++	protein +++	protein +++	protein ++	protein –
USG	1.036	1.036	1.016	1.042	–
Cytology	NSF	–	NSF	NSF	–
Microscopy	NSF	rod +++	NSF	NSF	–
UPCR	22.4	–	–	3.3	0.3

NSF = not specific factor; UPCR = urine protein creatinine ratio; USG = urine specific gravity

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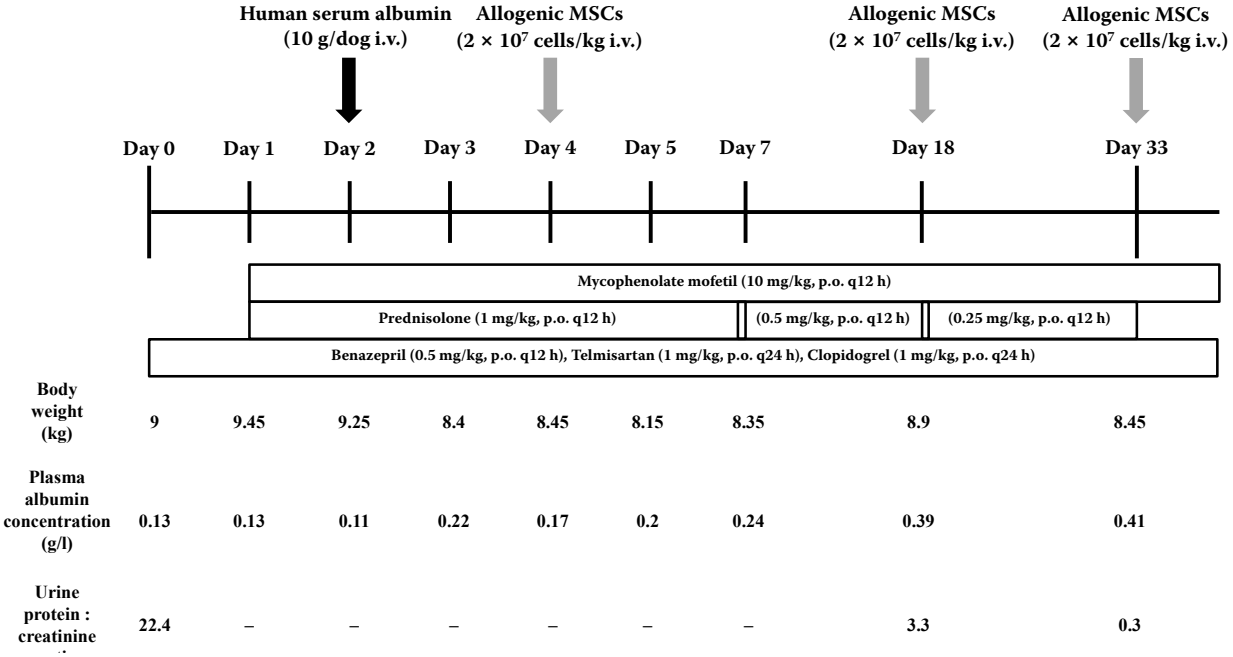

Figure 1. The overall process

nificantly enough to eat on its own, which led to the conversion of the patient to outpatient treatment.

Tapering of glucocorticoid began upon discharge form the hospital because prednisolone was not thought to be an effective drug for this patient. Furthermore, the patient's condition gradually improved even after converting to outpatient treatment. We decided to continue the stem cell therapy every two weeks at least six times. After the mesenchymal stem cells were injected into the patient and over the course of 33 days, the plasma albumin concentration gradually increased, and the urine protein to creatinine ratio was reduced from 22.4 to 0.3. Table 1 describes the urinalysis of the patient, and Figure 1 depicts the overall process up to this point. All of the medical treatments were stopped 46 days after the initial hospital visit, but the stem cell injections were conducted total six time of infusion. The patient maintained a healthy condition, and NS did not recur without medical treatment for 1 year.

DISCUSSION

NS is defined by the simultaneous presence of four clinical features – proteinuria, hypoalbuminaemia, extravascular fluid accumulation, and hyperlipidaemia. This disease usually progresses, gradually, and the prognosis of this condition in dogs is poor (Klosterman and Pressler 2011).

In dogs with NS, the use of immunosuppressive drug is controversial because they have numerous side effects including gastrointestinal upset, myelosuppression, pancreatitis, and hepatotoxicity; moreover, the evidence supporting this therapeutic practice in dogs is lacking (Segev et al. 2013). However, immunological actions are thought to play a significant role in the mechanism of glomerular disease. For example, immunocomplex deposition in glomeruli may decrease the amount of fixed negative charge in the glomeruli and complement activation, which results in membrane damage; thus, immunosuppressive therapy seems to be a logical candidate for the treatment of glomerular disease (van den Berg and Weening 2004; Pressler et al. 2013; Segev et al. 2013).

In severe cases of NS where immediate immunosuppression is required, the short-term administration of glucocorticoids seems appropriate if their use is adjusted to minimise their adverse effects (Center et al. 1987). In this case, the glucocorticoid dose was reduced at 6 days due to a lack of clinical improvement and concerns about its side effects. Mycophenolate mofetil is commonly used in human medicine to treat glomerular disease (Appel and Appel 2009). It is recommended as a treatment option for per-acute or rapidly progressive glomerular disease with or without histopathological confirmation because of its low risk of complications (Banyard and Hassett 2001). In this study, the

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patient's condition was too poor to conduct a renal biopsy, but the hypoalbuminaemia and proteinuria worsened gradually. Thus, the use of mycophenolate mofetil was started without a histopathological examination; however, no significant negative effects of this drug were observed.

Furthermore, allogeneic mesenchymal stem cells have been shown to aid in tissue restoration and immune regulation (Kode et al. 2009; Teng et al. 2015). Taking these effects into consideration, numerous studies on the use of mesenchymal stem cells in kidney diseases as well as clinical trials in human and veterinary medicine are being conducted (Quimby et al. 2013; Peired et al. 2016; Lee et al. 2017; Bochon et al. 2019).

A previous study reported that if mesenchymal stem cells are injected directly into the kidney in a canine acute kidney disease model, prolongation of the median survival time can be obtained (Lee et al. 2017). Several studies have shown the effectiveness of intravenous administration of mesenchymal stem cells in kidney diseases in cats (Quimby et al. 2013). Based on these factors, allogeneic adipose tissue-derived mesenchymal stem cells were injected intravenously in the present patient. In the present study, using cryopreserved allogeneic mesenchymal stem cells was advantageous regarding time and costs. Cultivating mesenchymal stem cells from autologous tissues in the patient is time-consuming and expensive, and there are risks regarding failure of successful cultivation. Most of all, removing some tissues surgically for stem cell culture in deteriorated patients is impossible. Furthermore, because donor conditions can affect the stem cell quality, allogeneic mesenchymal stem cells can be isolated from patients whose health cannot be guaranteed (Lukomska et al. 2019).

In this study, immunosuppressant drugs and mesenchymal stem cells were used to treat the NS condition in a dog, where the NS successfully improved over time. The combination therapy of immunosuppressant drugs and mesenchymal stem cells has not been widely used, and its clear mechanism and efficacy are unknown. However, previous studies have shown that some immunosuppressive drugs increase the function of the mesenchymal stem cells (Schneider et al. 2015). Moreover, mesenchymal stem cells can also improve the efficacy and reduce the side effects of immunosuppressive therapy (Lee et al. 2018).

There are some limitations to this study. As we used both mesenchymal stem cells and immunosuppressive treatment due to the patient's critical condition, it is difficult to determine which treatment had a significant effect on the patient. Although the exact action of these treatments to each therapy was not clear, in this case, the NS was resolved after the co-treatment with mesenchymal stem cells and immunosuppressive drugs. If more data are gathered and studies are conducted, we will be able to prove the effectiveness of the mesenchymal stem cells co-administration with immunosuppressant drugs in glomerular diseases.

In conclusion, it could be stated that glomerular disease with NS in young dogs is uncommon and difficult to treat. In this study, we simultaneously used immunosuppression therapy and allogeneic mesenchymal stem cells as a trial treatment, and no significant side effects were observed, resulting in a clinical cure. This case report is a good example of the use of immunosuppressive medication and allogeneic mesenchymal stem cells in treating glomerular disease with NS in dogs.

Conflict of interest

The authors declare no conflict of interest.

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