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Improvement of systemic lupus erythematosus in dogs with canine adipose-derived stem cells

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Abstract: A 6-year-old, intact female, Maltese presented with limited movement of the hind limbs and intermittent pruritus for three months. The patient was diagnosed with systemic lupus erythematosus. Conventional immunosuppressive therapy was attempted for 70 days; however, the patient still suffered from life-threatening pancreatitis and hepatopathy. Therefore, we tried canine adipose-derived mesenchymal stem cells for immunomodulation and liver protection. After 6-months of the stem cell therapy, the patient's walking and hepatopathy improved. These findings indicate that stem cell therapy may be another option for systemic lupus erythematosus in dogs.

Keywords: dog; SLE; stem cell therapy

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects the joints, skin, kidneys, and many other organ systems. The most common clinical signs of SLE are fever, lameness by polyarthritis, dermatological manifestations, petechiae, or bruising caused by thrombocytopenia, immune-mediated haemolysis, and kidney failure because of glomerulonephritis (Fournel et al. 1992). The antinuclear antibody (ANA) test and lupus erythematosus (LE) test are used for the diagnosis of SLE. The LE test is highly specific for SLE, but rarely used clinically because of its lack of sensitivity. The ANA test is more sensitive and useful in the diagnosis of SLE (Costa et al. 1984).

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Many criteria for the diagnosis of SLE in dogs have been derived from human medicinal literature, and the most commonly accepted criteria are cutaneous lesions, polyarthritis, polymyositis, haemolytic anaemia, glomerulonephritis, leukopenia, thrombocytopenia (major signs), fever of unknown origins, neurological disorders, oral ulcers and pericarditis, and pleuritis (minor signs) (Jones 1993). For the diagnosis of SLE, at least two major signs and a high serum ANA titre should be present (Ettinger and Feldman 2005). Immunosuppression is essential for the treatment of SLE; however, immunosuppressive drugs can have adverse effects, such as bone marrow suppression, gastrointestinal disorders, pancreatitis, and hepatotoxicity (Fournel et al. 1992). Here, we describe that canine adipose-derived stem cell treatment in a dog with systemic lupus erythematosus, which had severe side effects with previous immunosuppressive drugs.

Case description

A 6-year-old, intact Maltese female weighing 3.8 kg in body weight presented with limited movement of the hind limbs and intermittent pruritus for three months. The physical examination revealed multiple, symmetrical joint swelling and erythematous lesions over the whole body. The complete blood count, serum biochemistry, and electrolyte profiles were within the reference ranges (IDEXX Reference Laboratories, Westbrook, ME, USA). The screening of tick-borne diseases (snap 4DX plus test, IDEXX, Seoul, Republic of Korea) was negative. The radiographic imaging revealed the soft tissue swelling of the joint, collapsed joint space, and subchondral bone erosions in the carpus. The femoral trochlear groove and patella showed erosive change and both stifle joints were luxated (Figure 1A, 1B). Synovial fluid was collected via arthrocentesis from the left stifle joint. The aerobic and anaerobic bacterial culture tests of the synovial fluid were negative and the synovial cell count revealed that non-degenerative neutrophils typically accounted for most of the cells. Erythematous lesions over the whole body were present, but no evidence of infectious agents was found in the skin screening test. The urine specific gravity was over 1.050 and the urine protein to creatinine ratio was 1.56. A urine sediment examination and urine bacterial culture test con-

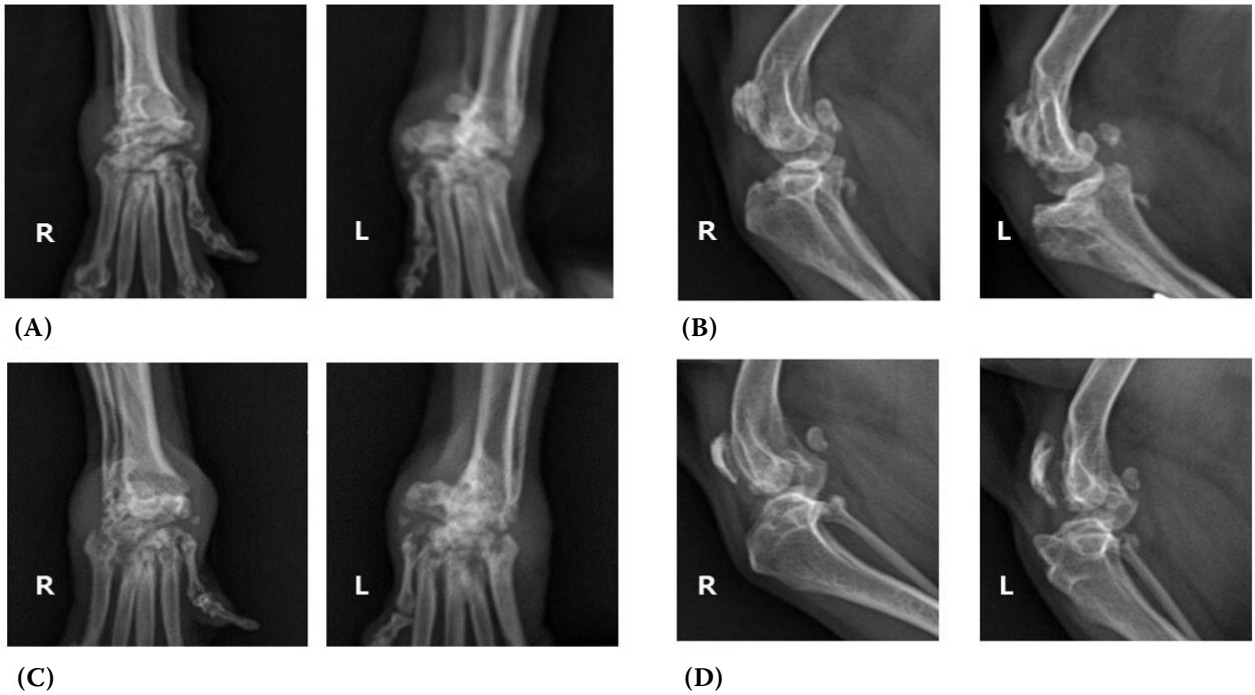

Figure 1. (**A**) The carpal joint before the stem cell treatment. The subchondral bone erosions and joint swelling are visible. (**B**) The stifle joint before the stem cell treatment. Femoral trochlear groove's and patella's erosive changes are visible. Patella luxation is also visible. (**C**) The carpal joint after the stem cell treatment. There is no radiological improvement after the stem cell therapy. (**D**) The stifle joint after the stem cell treatment. There is no radiological improvement after the stem cell therapy

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firmed that there was no evidence of a lower urinary tract infection. Therefore, the proteinuria of this patient was assumed to not be caused by lower urinary tract infections, but by glomerulonephritis. The ANA test was positive (1 : 200) (IDEXX Reference Laboratories, Westbrook, ME, USA) and the patient had three major signs compatible with SLE (polyarthritis, skin lesions, glomerulonephritis); therefore, this patient was diagnosed with SLE.

The initial treatment was prednisolone (2 mg/kg, oral administration (*p.o.*), every 12 h, b.i.d.), azathioprine (2 mg/kg, *p.o.*, every 24 h, s.i.d.), misoprostol (3 µg/kg, *p.o.*, b.i.d.), famotidine (0.5 mg/kg, *p.o.*, b.i.d.), antibiotics (cephalexin (25 mg/kg, *p.o.*, b.i.d.) and hepatoprotective drugs (silymarin 10 mg/kg, *p.o.*, b.i.d., S-adenosyl methionine 192 mg, *p.o.*, s.i.d., ursodeoxycholicacid 10 mg/kg, *p.o.*, b.i.d.). Despite treatment continuing for 70 days, no significant improvements in the clinical symptoms were observed. Rather, after the conventional immunosuppressive therapy, the patient was presented with abdominal distension, acute vomiting and severe abdominal pain. The patient also showed elevated liver enzyme activities and hepatomegaly and was canine pancreas-specific lipase (SNAP® cPL, IDEXX, Seoul, Republic of Korea) positive. Based on these findings, we diagnosed the patient with hepatotoxicity and having acute pancreatitis caused by the immunosuppressive drugs administration. Therefore, the patient was hospitalised for 14 days for treatment of the hepatotoxicity and acute pancreatitis. Although the doses of prednisolone and azathioprine were gradually tapered, the liver enzyme activities, abdominal distension and hepatomegaly did not improve for one month.

After obtaining the owner's consent, a stem cell therapy was conducted with the expectation of immunomodulation and liver protection. In order to obtain the stem cells, two intact Beagle female dogs were sedated with midazolam (0.1 mg/kg) and acepromazine (0.03 mg/kg) and then anaesthetised with isoflurane (0.25–3% isoflurane in 100% oxygen in an induction chamber). A 2–4 g sample of the ventral midline subcutaneous fat from each animal was obtained and collected in a phosphate buffered saline (PBS, Gibco, Thermo Fisher Scientific, MA, USA). This study was approved by the Kangwon National University Institutional Care and Animal Use Committee (KW-160809-1). Each tissue sample was minced into small pieces with a sterile scalpel blade and broken down further with 0.075% of Collagenase I (Gibco, Thermo Fisher Scientific, MA, USA) in HBSS (Hank's Balanced Salt Solution) (Sigma-Aldrich, St. Louis, MO). The cells obtained from the broken-down tissue were resuspended in 5 ml DMEM (Dulbecco's Modified Eagle Medium) (Gibco, Thermo Fisher Scientific, MA, USA) with 5% FBS (Fetal Bovine Serum) and plated in T-25 plastic tissue culture flasks. After reaching 80% confluency, the cells were resuspended in 20 ml of the medium and divided into 2 T-75 plastic tissue culture flasks. All the cell cultures went through the same procedure until the third passage of the cells was available for the characterisation. The isolation and characterisation procedure of the adipose-derived stem cells followed that of the previous study (Ko et al. 2018).

In the first two trials, the stem cells (5.0×10^6 cells) were injected intravenously at two-week-intervals. During the first two months of treatment, the elevated liver enzyme activities started to decrease dramatically after the intravenous initiation of the stem cell therapy in the SLE patient, after which the patients' condition stabilised and

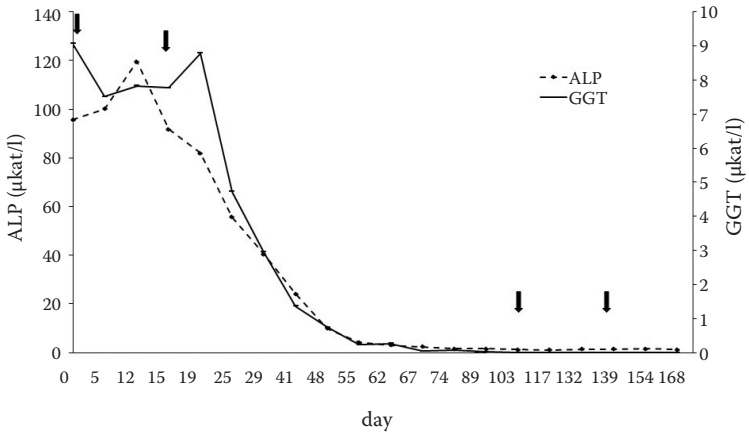

Figure 2. A graph of the change in the liver enzyme activity from the day of the adipose derived MSCs (mesenchymal stem cells) injection to 250 days, the ALP (alkaline phosphatase) and GGT (gamma glutamyltransferase) activities. At first presentation, all the liver enzyme activities were markedly increased: ALP 95.62 µkat/l (Reference range, 0.13–1.67 µkat/l) and GGT 9.07 µkat/l (Reference range, 0–0.23 µkat/l). During the adipose derived MSCs therapy, the liver enzyme activity gradually decreased. The arrows indicate the time of the adipose-derived MSC injections

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its appetite and vitality improved (Figure 2). At 5 months after the second stem cell intravenous injection, the owner reported that the patient showed continuous pain in the joint area. Two additional treatments (the third and fourth injections) were administered via intravenous and intra-articular injection (5.0×10^6 cells injection into the left carpal joint cavity and the right stifle joint cavity, respectively). Three weeks after the fourth stem cell administration, the patient began to walk, and gait improvement was observed following the subsequent treatment even though there was no improvement on the radiography (Figure 1C, 1D).

DISCUSSION

While administration of immunosuppressive drugs to dogs is critical to control of immune-mediated disease, the administration of these drugs can cause adverse effects. In this study, a patient diagnosed with SLE showed adverse effects including hepatic insufficiency and acute pancreatitis to immunosuppressive drugs. Among the many types of immunosuppressive agents, prednisolone and azathioprine are commonly used to treat dogs; however, they may have adverse effects (Wallisch and Trepanier 2015). The most common side effects of glucocorticoids are polyuria, polydipsia, polyphagia, gastrointestinal haemorrhage and thromboembolism, while the most common adverse effects of azathioprine are liver toxicity, bone marrow suppression, and acute pancreatitis (Wallisch and Trepanier 2015). In humans, the incidence of azathioprine-induced hepatotoxicosis ranges from 4% to 24% depending on the patient population; however, treatment only needs to be discontinued in 3–4% of these patients (Bastida et al. 2005; Gisbert et al. 2007). In dogs, the incidence of azathioprine induced hepatotoxicosis was about 15% (Wallisch and Trepanier 2015).

In human medicine, adverse effects of long-term use of immunosuppressive drugs have been reported to increase the mortality rate. Therefore, in patients with SLE, therapeutic studies using stem cells are actively conducted (Phillips et al. 2017). In veterinary medicine, there is a growing interest in the treatment of regenerative and intractable diseases using mesenchymal stem cells (MSCs). MSCs derived from adipose tissue have recently been actively investigated because of the ease of the tissue collection and availability of the adipose tissue in various parts of the body (Stewart and Stewart 2011). MSCs, which are known to have immunomodulatory properties, can differentiate into various cell types. Moreover, studies have reported the immunoregulatory ability of adipose derived MSCs in the treatment of feline asthma (Trzil et al. 2016) and canine keratoconjunctivitis sicca (Villatoro et al. 2015), and MSCs have been shown to have an antioxidant effect on damaged hepatocytes (Quintanilha et al. 2014).

There are several routes to the transplantation of MSCs, including the direct injection to the target organ and intravenous systemic injection. However, it is still not clear which route of administration is the most effective. A previous study showed that when foetal liver stem cells were transplanted into mice, the intraportal route more effectively targeted the liver than the intrasplenic route (Cheng et al. 2009). However, Kim et al. (2011) found that the systemic circulation can exert endocrine and paracrine effects to ameliorate the injured liver. In addition, the results of a previous study suggest that direct intraarticular injection has a significant therapeutic effect in patients with induced articular cartilage damage (Mokbel et al. 2011).

In this case report, the patient suffered from liver damage because of the adverse effects of the immunosuppressive therapy for the SLE treatment. Since the patient had not responded to the conventional treatment and had experienced liver function deterioration, the other treatments using adipose derived MSCs was selected. Even without any other immunosuppressive therapy, other than adipose derived MSCs treatment, the patient was found to have a significant decrease in the liver enzyme levels. It is not clear whether the sudden improvement of the clinical signs (e.g., beginning to walk) was due to the intra-joint cavity injection of the adipose derived MSCs alone. However, it is conceivable that the injection of the adipose derived MSCs into the joint cavity had positive effects on the patient. This result showed that stem cell therapy may be another option for systemic lupus erythematosus in dogs.

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