pISSN 1598-298X
J Vet Clin 31(3) : 253-257 (2014)

Journal of Veterinary Clinics
www.ksvc.or.kr

Clinical Application of Autologous Adipose Tissue Derived Mesenchymal Stem Cells in Five Dogs with Stifle Joint Osteoarthrosis

Hun-Young Yoon*, Dong Jun Kang, Soo-Han Lee, Soon-wuk Jeong*, and Byung-Hyun Chung[1]

*Department of Veterinary Surgery and *Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea*

(Accepted: April 18, 2014)

Abstract : Five dogs presented with a history of pelvic limb lameness. On physical examination of the stifle joints, five dogs had pain, lameness, patellar luxation, or ligamentous instability. Craniocaudal and mediolateral radiographic projections revealed osteophytes or subchondral cystic lesions on the stifle joints. Based on a previously described Osteoarthrosis (OA) scoring technique, five dogs showed high OA scores. Combination of surgery and implantation of autologous adipose tissue derived mesenchymal stem cells (aAT-MSCs) or percutaneous injection of aAT-MSCs was determined with informed consent. 1×10^6 aAT-MSCs suspended in PBS was injected in the stifle joints. The follow-ups were completed 12 months after surgery. The follow-up information was based on physical examination by veterinarians. The lameness, pain on manipulation, and OA scores improved six or 12 months after implantation of aAT-MSCs. There was a radiographic evidence of decreased osteophytes and subchondral cystic lesions. These results suggest that implantation of aAT-MSCs can be considered an option for management of cases of OA in the stifle joints.

***Key words* :** autologous adipose tissue-derived stem cells, stifle joint, osteoarthrosis, dog.

Introduction

Osteoarthrosis (OA) is recognized as a significant health problem affecting domestic dogs worldwide (15). It has been estimated that 20% of adult dogs have OA (9). The disease is characterized by progressive damage to the articular cartilage, changes in the underlying (subchondral) bone, and occasional mild synovial inflammation (3,12,16). OA is a chronic, disabling condition, and until now, no regenerative therapeutic options have been available to prevent, delay, halt, or heal OA (16,20). In contrast to bone, skin, liver, or muscle, cartilage seems to be devoid of an internal tissue-specific stem cell compartment and shows a very limited capacity for tissue homeostasis and regeneration in response to damage (1,20). Currently available options for OA management include joint lavage, tissue debridement, nonsteroidal anti-inflammatory drugs (NSAIDs), nutraceuticals, microfracture of the subchondral bone, or transplantation of osteochondral grafts; however, scientific studies and clinical experience with OA management in dogs suggest that the aforementioned options often do not provide satisfactory outcomes (5,8).

Autologous adipose tissue-derived mesenchymal stem cells (aAT-MSCs) are an abundant population of adult stem cell that is readily isolated from canine adipose tissue. These cells are a potential source for the repair of cartilage defects because of their multipotency (differentiation into bone, muscle, fat, and cartilage) and practical access. The purpose of this case series is to describe the effect of aAT-MSCs on orthopedic function in dogs with stifle joint OA.

Case

A 12-year-old castrated male English Cocker Spaniel weighing 15.4 kg presented to The Woosung Animal Hospital with 5-year history of bilateral pelvic limb lameness. The owner reported that the lameness was not resolved with a combination of non-steroidal anti-inflammatory drugs, steroids, and exercise restriction. A month prior to presentation, the dog was intermittently non-weight bearing on the right pelvic limb. Physical examination of the stifle joints revealed pain and a grade 2 medial patellar luxation (manual displacement of the patella with pressure and reposition with animal extension or tibial derotation in the trochlear groove) on the bilateral pelvic limbs. The right and left stifle joints were associated with a lameness of grade 3 and grade 2 (0 = no appreciable lameness, 1 = intermittent weight-bearing lameness, 2 = obvious weight-bearing lameness, 3 = intermittent non-weight-bearing, 4 = obvious non-weight-bearing), respectively. Tests for ligamentous instability revealed nothing remarkable. Craniocaudal and mediolateral radiographic projections revealed osteophytes or subchondral cystic lesions on the right and left stifle joints (Fig 1). Based on a previously described OA scoring technique (13), an OA score for each stifle was determined by evaluating 32 specific radiographic features of OA. Osteoarthrosis scores for the right and left stifle joints

[1]Corresponding author.
E-mail : chungbh@konkuk.ac.kr

Fig 1. Pre-injectional craniocaudal radiograph from the stifle joint of a 12-year-old dog with an OA score of 32 (right) and 29 (left), respectively. The patient's right is on the left.

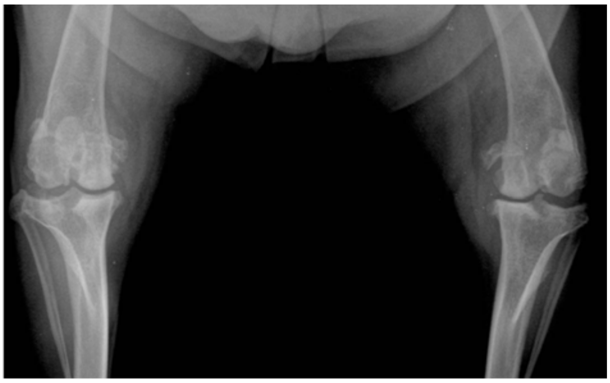

Fig 2. Six months post-injectional craniocaudal radiograph from the stifle joint of a 12-year-old dog with an OA score of 22 (right) and 18 (left), respectively. The patient's right is on the left.

were 32 and 29 respectively. A diagnosis of OA on the right and left stifle joints with a grade 2 medial patellar luxation was made. Percutaneous implantation of aAT-MSCs was determined for the treatment of bilateral OA with informed consent.

Seven days later, to isolate the aAT-MSCs, adipose tissue was collected from the subcutaneous fat of the dorsal pelvis under general anesthesia and aseptic conditions. The adipose tissue was then washed with phosphate buffered saline (PBS) to remove debris and excess blood, after which it was dissected. Minced tissue was then placed into a fat processing unit (AdipoSys®, Equipforstem, South Korea) with an equal volume of pre-warmed PBS, followed by 45-second vortex agitation. The mixture was centrifuged at 4000 rpm for 8 minutes producing 3 phases: oil, squeezed fat, and blood parts phases. Oil and blood parts were discarded. To isolate cells, an equal volume of enzyme (AdipoZyme®, Equipforstem, South Korea) was then added to the squeezed fat, followed by 5-minutes vortex agitation. The digested tissue was subsequently placed in an incubator (AdipoCulti®, Equipforstem, South Korea) at 37°C for 30 minutes until the tissue became homogenous. The mixture was placed in 15-second vortex agitation and centrifuged at 200 g for 4 minutes. The supernatant was discarded. The cell pellet was collected and resuspended with PBS. The mixture was then placed in15-second vortex agitation and centrifuged at 300 g for 5 minutes. The supernatant was discarded. These procedures including resuspension, agitation, centrifuge, and discarding were repeated three times. Cell counting was performed using a hemocytometer (Countess™, Invitrogen™, South Korea) after trypan blue staining.

Percutaneous implantation of aAT-MSCs was performed. The patient was positioned in lateral recumbence. The right and left stifle joints area was aseptically prepped with povidone-iodine, alcohol, and sterile drapes. The stifle joints were then bent at a 45° angle. A 24-gauge needle was inserted through a craniomedial approach. Once the joint space was attained, 1×10^6 aAT-MSCs suspended in PBS were

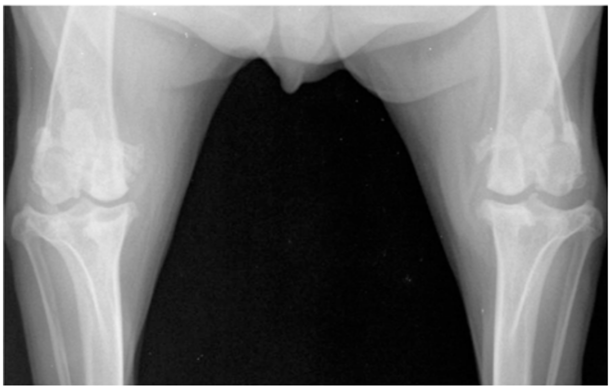

Fig 3. One year post-injectional craniocaudal radiograph from the stifle joint of a 12-year-old dog with an OA score of 15 (right) and 9 (left), respectively. The patient's right is on the left.

injected. The owner was instructed that the patient remained still for a few hours to allow for cell attachment and maintained activity as tolerated.

The patient follow-up was performed by a veterinarian for a year after treatment. Each stifle joint was subjectively evaluated, based on history and orthopedic examination for degree of lameness and assigned a grade. The lameness grade and osteoarthrosis scores of the right and left stifle joints were 1 and 1 and 22 and 18, 6 months after treatment, respectively. On a mediolateral radiographic projection and a craniocaudal radiographic projection, a decrease in osteophytes and subchondral cystic lesions was evident 6 months and a year after treatment on the bilateral stifle joints (Figs 2 and 3). The lameness grade and osteoarthrosis scores of the right and left stifle joints were 1 and 0 and 15 and 9, a year after treatment, respectively. There was no evidence of pain on the bilateral stifle joints a year after treatment.

During the period of 2011, isolation and implantation of aAT-MSCs was performed at Konkuk Biotechnology Co., Ltd., (KKBT) in a total of five dogs with stifle joint OA including the case detailed in this report. Signalment, whether

Table 1. Signalment, whether there is patellar luxation or ligamentous instability, pain, lameness grade, and osteoarthrosis (OA) scores in five dogs with implantation of autologous adipose tissue-derived mesenchymal stem cells

Case No.	Breed	Age (year)	Sex	Weight (kg)	Patellar luxation	Ligament instability	Injection site	Pain			Lameness grade			OA scores		
								BT	6 ms AT	1 y AT	BT	6 ms AT	1 y AT	BT	6 ms AT	1 y AT
1	Pomeranian	2	IM	2.98	L (G4)	N	LSJ	Y	N	N	3	0	0	10	8	7
2	Pomeranian	1	CM	3.38	R (G3)	N	RSJ	Y	N	N	3	0	0	11	7	5
3	Maltese	9	CM	3.8	L (G3)	Y	LSJ	Y	N	N	3	1	0	23	14	12
4	Maltese	7	CM	3.1	N	N	RSJ	Y	Y	Y	4	4	4	20	18	17
5	ECS	12	CM	15.4	R (G2)	N	RSJ	Y	Y	N	3	1	1	32	22	15
							LSJ	Y	Y	N	2	1	0	29	18	9

BT, Before Treatment; ms, months; AT, After Treatment; y, year; ECS, English Cocker Spaniel; IM, Intact Male; CM, Castrated Male; L, Left; G, Grade; R, Right; N, No; Y, Yes; LSJ, Left Stifle Joint; RSJ, Right Stifle Joint

there is patellar luxation or ligament instability, pain, lameness grade, and OA scores are summarized in the Table 1. Implantation of aAT-MSCs was performed after surgical reduction for patellar luxation (n = 2) or for both patellar luxation and ligamentous instability (n = 1) in three cases. For the two cases, percutaneous implantation was performed without surgical treatment. No improvement of pain and lameness grade was evident in one case where surgical reduction for patellar luxation was performed two years ago. Bilateral implantation of aAT-MSCs was performed in one case. The follow-ups were completed one year after surgery. The follow-up information was based on physical examination by veterinarians.

Discussion

Growing interest in cell therapy using adult stem cells has attracted the attention of the biomedical research fields including those studying OA. In humans, use of aAT-MSCs has been described to manage case of long-term knee pain (4). In this case report, there was MRI evidence of increased meniscus volume and femoral cartilage volume at three months after percutaneous implantation of aAT-MSCs. The use of aAT-MSCs has also been described for the management of dogs with chronic OA of the coxofemoral joint or the stifle joint (2,21). Improvement of ambulatory function was evident based on history, physical examination, and lameness examination after the implantation of aAT-MSCs to the hip joint or stifle joint. In the present case series, there was evidence of improvement of pain, ambulatory function, and OA scores in response to treatment with aAT-MSCs in four dogs. These results showed that aAT-MSCs could be effective to prevent, delay, halt, or heal OA.

In the present case series, aAT-MSCs suspended in PBS were implanted into the stifle joint with a percutaneous intra-articular injection technique in two dogs without surgical treatment and with a combination of surgical treatment in three dogs, respectively. With a percutaneous injection technique, implantation of aAT-MSCs was conducted without joint capsulotomy, which resulted in no pain secondary to the surgical procedure, no surgical complications such as soft tissue swelling, and a shorter recovery period. In addition, percutaneous implantation is a non-invasive method that allows a return to normal daily life immediately after implantation of aAT-MSCs. As such, percutaneous intra-articular injections might be advantageous for use in aging OA patients and those with diabetes mellitus or chronic diseases that pose a high anesthetic risk since this technique is performed without general anesthesia.

In spite of growing interest of cell therapy, veterinarians may be reluctant to perform regenerative medicine technique clinically due to the technical concerns associated with tissue engineering. In tissue engineering, stem cells are locally implanted with a scaffold to replace or enhance tissue function that has been impaired by disease, injury, or age (7,14); whereas, in the present case series, aAT-MSCs suspended in PBS were injected into the stifle joint in an attempt to impart their benefit to broad area in the stifle joint. Tissue engineering requires technical considerations including seeding a biomaterial scaffold with cells, genetic matching (if the patient's own cells are not an option), and selecting or creating an appropriate scaffold to facilitate cell attachment, permit diffusion of nutrients from the blood, and mimic the mechanical properties of the tissues (14). In the present case series, aAT-MSCs were easily injected into the stifle joint using a syringe without any special considerations, which suggests that application of regenerative medicine might be more practical for clinical use.

The present case series showed that aAT-MSC therapy resulted in improved orthopedic examination scores. The lameness, pain on manipulation, and OA scores improved over time. Additionally, the present case series provided radiographic evidence of decreased osteophytes and subchondral cystic lesions; however, no improvement of pain and lameness grade was evident in one case. Osteoarthrosis is related to a variety of causes including degenerative joint disease, septic arthritis, viral arthritis, immune-mediated arthritis, trauma, neoplasia, and so on (6). These diseases have a different etiology and severity of pathologic changes, which can effect on outcome after implantation of aAT-MSCs. A study is warranted to determine the effect of aAT-MSCs, depending on causes resulting in OA, on patients with stifle joint OA.

The purpose of OA treatment is to reduce pain, increase function, and improve overall symptoms (17). Previous studies have reported that delivery of stem cells to joints with OA enhances the repair of damaged tissue or impedes progression to OA (17-19). In the present case series, a decrease of pain was evident at six or 12 months after injection of aAT-MSCs. Therefore, it is possible that an increase in ambulation could be attributed to a decrease of pain secondary to OA.

Previous studies have reported that the therapeutic effects of aAT-MSCs on stifle joints with OA are attributable to the placement of cells in the defect, their capacity to differentiate into cartilage, tendon, muscle, bone, and adipose tissue, the enhancement of cartilage regeneration, and the stimulation of cell division after local delivery of aAT-MSCs to lesions of the cartilage, subchondral bone, ligaments, joint capsule, synovial membrane, and periarticular muscles (17-19). These therapeutic effects of aAT-MSCs might be affected by platelets since platelets contain critical growth factors and mediators of tissue repair pathways (4,10). In response to tissue injury, a complex cascade of cellular and noncellular signals triggers platelet receptors, resulting in expulsion of these growth factors within the site of injury (4,11). In the present case series, there was evidence of a decrease in osteophytes and subchondral cystic lesions without the use of platelet rich plasma. The aAT-MSCs might be responsible for secreting mitosis-stimulating factors and/or growth factors. Further study is warranted to better determine the effect of these cells on the secretion of mitosis-stimulating factors and/or growth factors.

Since implantation of aAT-MSCs provided excellent improvement in lameness, pain, osteophytes, and subchondral cystic lesions in dogs with stifle joint OA, this treatment can be considered an option for management of stifle joint OA in dogs or for those undergoing surgical management for CrCL injury or patellar luxation lesions to impede the progression to OA which is typical consequence of such injuries.

Acknowledgements

This paper was written as part of Konkuk University's Research Support Program for its faculty on sabbatical leave in 2013.

References

1. Anderst WJ, Les C, Tashman S. In vivo serial joint space measurements during dynamic loading in a canine model of osteoarthritis. Osteoarthritis Cartilage 2005; 13: 808-816.
2. Black LL, Gaynor J, Gahring D, Adams C, Aron D, Harman S, Gingerich DA, Harman R. Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter, controlled trial. Vet Ther 2007; 8: 272-884.
3. Boyd SK, Müller R, Matyas JR, Wohl GR, Zernicke RF. Early morphometric and anisotropic change in periarticular cancellous bone in a model of experimental knee osteoarthritis quantified using microcomputed tomography. Clin Biomech 2000; 15: 624-631.
4. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. Pain Physician 2008; 11: 343-353.
5. Estes BT, Wu AW, Guilak F. Potent induction of chondrocytic differentiation of human adipose-derived adult stem cells by bone morphogenetic protein 6. Arthritis Rheum 2006; 54: 1222-1232.
6. Fossum TW. Disease of the joints. In: Small animal surgery, 3rd ed. St. Louis: Mosby. 2007: 1155-1170.
7. Gimeno MJ, Maneiro E, Rendal E, Ramallal M, Sanjurjo L, Blanco FJ. Cell therapy: a therapeutic alternative to treat focal cartilage lesions. Transplant Proc 2005; 37: 4080-4083.
8. Hielm-Björkman A, Tulamo RM, Salonen H, Raekallio M. Evaluating Complementary Therapies for Canine Osteoarthritis Part I: Green-lipped Mussel (Perna canaliculus). Evid Based Complement Alternat Med 2009; 6: 365-373.
9. Johnston SA. Osteoarthritis. Joint anatomy, physiology, and pathobiology. Vet Clin North Am Small Anim Pract 1997; 27: 699-723.
10. Khan WS, Tew SR, Adesida AB, Hardingham TE. Human infrapatellar fat pad-derived stem cells express the pericyte marker 3G5 and show enhanced chondrogenesis after expansion in fibroblast growth factor-2. Arthritis Res Ther 2008; 10: R74.
11. Kilroy GE, Foster SJ, Wu X, Ruiz J, Sherwood S, Heifetz A, Ludlow JW, Stricker DM, Potiny S, Green P, Halvorsen YD, Cheatham B, Storms RW, Gimble JM. Cytokine profile of human adipose-derived stem cells: expression of angiogenic, hematopoietic, and pro-inflammatory factors. J Cell Physiol 2007; 212: 702-709.
12. Lahm A, Uhl M, Edlich M, Erggelet C, Haberstroh J, Kreuz PC. An experimental canine model for subchondral lesions of the knee joint. Knee 2005; 12: 51-55.
13. Lazar TP, Berry CR, deHaan JJ, Peck JN, Correa M. Long-term radiographic comparison of tibial plateau leveling osteotomy versus extracapsular stabilization for cranial cruciate ligament rupture in the dog. Vet Surg 2005; 34: 133-141.
14. Lee JW, Kim YH, Kim SH, Han SH, Hahn SB. Chondrogenic differentiation of mesenchymal stem cells and its clinical applications. Yonsei Med J 2004; 45: 41-47.
15. Marshall WG, Hazewinkel HA, Mullen D, De Meyer G, Baert K, Carmichael S. The effect of weight loss on lameness in obese dogs with osteoarthritis. Vet Res Commun 2010; 34: 241-253.
16. Martel-Pelletier J, Lajeunesse D, Fahmi H, Tardif G, Pelletier JP. New thoughts on the pathophysiology of osteoarthritis: one more step toward new therapeutic targets. Curr Rheumatol Rep 2006; 8: 30-36.
17. Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. Arthritis Rheum 2003; 48: 3464-3474.

18. Quintavalla J, Uziel-Fusi S, Yin J, Boehnlein E, Pastor G, Blancuzzi V, Singh HN, Kraus KH, O'Byrne E, Pellas TC. Fluorescently labeled mesenchymal stem cells (MSCs) maintain multilineage potential and can be detected following implantation into articular cartilage defects. Biomaterials 2002; 23: 109-119.
19. Wakitani S, Yamamoto T. Response of the donor and recipient cells in mesenchymal cell transplantation to cartilage defect. Microsc Res Tech 2002; 58: 14-18.
20. Winter A, Breit S, Parsch D, Benz K, Steck E, Hauner H, Weber RM, Ewerbeck V, Richter W. Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells. Arthritis Rheum 2003; 48: 418-429.
21. Yoon HY, Lee JH, Jeong SW. Long-term follow-up after implantation of autologous adipose tissue derived mesenchymal stem cells to treat a dog with stifle joint osteoarthrosis. J Vet Clin 2012; 29: 82-86.

무릎 골관절증을 보이는 개에서 자가지방유래 중배엽성 줄기세포 치료 다섯 증례

윤헌영* · 강동준 · 이수한 · 정순욱* · 정병현[1]

건국대학교 수의과대학 수의외과학 교실, *수의내과학 교실

요 약 : 후지 파행 증상을 보이는 다섯 마리 개가 내원하였다. 무릎 관절 신체 검사에서 통증, 파행, 슬개골 탈구, 또는 인대 손상을 확인 하였다. 일반 방사선 사진에서 골증식체와 연골하 병변을 확인 하였고 골관절증 점수가 높은 것을 확인하였다. 수술적 교정과 자가지방 유래 줄기세포 치료의 혼합 방법 또는 자가지방 유래 줄기세포의 경피 주입 단독 방법을 보호자의 동의 하에 실시 하였다. 1×10^6 개의 줄기세포를 PBS와 함께 주입 하였다. 수술 후 확인은 수의사의 신체검사를 통해 12개월까지 실시 하였다. 주입 후 6개월 또는 12 개월에 파행, 통증, 골관절증 점수가 개선되었음을 확인 하였고 일반 방사선 사진에서도 골증식체와 연골하 병변이 줄어든 것을 확인 할 수 있었다. 본 증례들를 통해 자가지방 유래 줄기 세포 치료가 골관절증을 보이는 개에서 효과적인 방법임을 확인 할 수 있었다.

주요어 : 자가지방유래 중배엽성 줄기세포, 무릎 관절, 골관절증, 개