pISSN 1229-845X, eISSN 1976-555X
J. Vet. Sci. (2013), **14**(4), 495-497
http://dx.doi.org/10.4142/jvs.2013.14.4.495
Received: 14 Dec. 2011, Revised: 21 Aug. 2012, Accepted: 30 Aug. 2012

JOURNAL OF
Veterinary
Science

Case Report

Percutaneous transplantation of human umbilical cord-derived mesenchymal stem cells in a dog suspected to have fibrocartilaginous embolic myelopathy

Wook-Hun Chung[1], Seon-Ah Park[1], Jae-Hoon Lee[1], Dai-Jung Chung[1], Wo-Jong Yang[1], Eun-Hee Kang[1], Chi-Bong Choi[1], Hwa-Seok Chang[1], Dae-Hyun Kim[1], Soo-Han Hwang[2], Hoon Han[2], Hwi-Yool Kim[1,*]

[1]*Department of Veterinary Surgery, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea*
[2]*Seoul Cord Blood Bank, Histostem Co., Seongnam 462-807, Korea*

The use of human umbilical cord blood-derived mesenchymal stem cells for cell transplantation therapy holds great promise for repairing spinal cord injury. Here we report the first clinical trial transplantation of human umbilical cord (hUCB)-derived mesenchymal stem cells (MSCs) into the spinal cord of a dog suspected to have fibrocartilaginous embolic myelopathy (FCEM) and that experienced a loss of deep pain sensation. Locomotor functions improved following transplantation in a dog. Based on our findings, we suggest that transplantation of hUCB-derived MSCs will have beneficial therapeutic effects on FCEM patients lacking deep pain sensation.

Keywords: dog, fibrocartilaginous embolic myelopathy, human umbilical cord-derived mesenchymal stem cells, percutaneous transplantation, xenotransplantaion

Fibrocartilaginous embolic myelopathy (FCEM) is a common cause of ischemic myelopathy in dogs and is a syndrome of acute spinal cord infarction due to embolization of fibrocarctilageous materials identified as the nucleus pulposus of intervertebral discs [3,5,9,12]. The pathogenesis of FCEM is still unclear [5,9,11]. A definitive diagnosis of this disease is confirmed by histopathological evaluation; therefore, antemortem diagnosis of FCEM is based on patient history, clinical examination, Cerebrospinal fluid (CSF) analysis, Computed tomography (CT) data, and magnetic resonance imaging (MRI) findings [1,5,8,11,12]. MRI is especially helpful for making an antemoterm diagnosis of ischemic myelopathy and eliminating other causes of myelopathy including degenerative disc disease. This technique can also be used to visualize signal intensity changes indicative of ischemic myelopathy [1,5,8,11,12].

The prognosis of FCEM depends on ischemia severity and extent of the affected area [5]. Previous reports described negative prognostic factors of FCEM including a loss of deep pain sensation, CSF changes, spinal cord swelling, and lack of improvement within the first two weeks of disease onset [3,5,7]. The absence of deep pain sensation after any spinal cord injury is indicative of a poor prognosis and significantly associated with bilateral damage to the gray and white matter [2,7]. Therefore, FCEM patients lacking deep pain sensation were given a guarded prognosis in previous reports [7]. Furthermore, methods for treating cases of FCEM without deep pain sensation have not been reported.

In a previous study using rat models of SCI, treatment with human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) led to significant improvement of locomotor function [4]. hUCB-MSCs were also found to be capable of differentiating into neural cells *in vivo* [10]. In fact, our previous studies have demonstrated that hUCB-MSCs promote regenerative potential in cases of SCI [10].

We encountered a dog (7-year-old female cocker spaniel) that was referred to the Konkuk University Veterinary Teaching Hospital (Korea) with an acute onset of hindlimb paralysis in the absence of any history of trauma. Neurological examination revealed that the dog suffered from hindlimb paralysis. No postural reaction was seen in the bilateral hindlimb including a lack of paw positioning, hopping, and extensor postural thrust. Additionally, no voluntary urination was observed. Spinal reflexes were normal. Deep pain sensation in the dog was absented at the

*Corresponding author: Tel: +82-2-450-3664; Fax: +82-2-446-9876; E-mail: hykim@konkuk.ac.kr

first neurological examination.

Methylprednisolone succinate (Koreamypharm, Korea) was administered intravenously (30 mg/kg) to reduce secondary spinal cord damage. MRI (0.3T; Esaote, Italy) was performed 24 h after clinical onset of symptoms (Fig. 1). There was no evidence of disc extrusion. An intramedullary lesion involving the gray matter alone, appearing as an area of hypointensity from L2 and L3 in the transverse image, was found on the T2-weighted image. CSF analysis was performed as well and did not produce any remarkable findings. We tentatively diagnosed the dog with FCEM based on the clinical symptoms, MRI findings, and CSF analysis according to previous studies [1,5,8,12]. Dorsal laminectomy was performed on the dog from L1 to L3 to promote decompression. The spinal cord was significantly swollen. However, no evidence of disc material extrusion or hemorrhage was found during surgery.

Deep pain sensation and motor function did not improve until 1 week after surgery. We notified the owners of the dog about the possibility of an adverse prognosis given the absence of deep pain sensation and significant spinal cord swelling. Participation of the dog in this clinical trial involving hUCB-derived MSC transplantation was offered and owner gave consent. Before transplantation, sensory evoked potential (SEP) analysis was performed (Sierra Wave, 2006; Cadwell Laboratories, USA). Based on the results, we confirmed that the dog did not show SEPs.

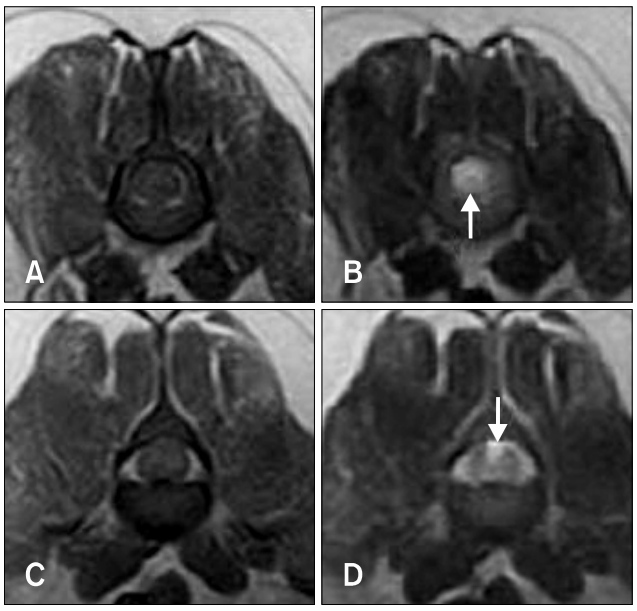

Fig. 1. T1-weighted (TR 530, TE 26) and T2-weighted (TR 3500, TE 90) MRI images of the dog 12 h (A～D) after the onset of clinical signs. The transverse T1-weighted image showed isointensity in the parenchyma of L2 (A) and L3 (C). Arrows: transverse T2-weighted image showing hyperintensity in the parenchyma of L2 (B) and L3 (D).

Transplantation of hUCB-derived MSCs was performed 7 days after decompression surgery. The hUCB-derived MSCs were provided for pure research purposes by the Seoul Cord Bank (Histostem, Korea). For the transplantation procedure, 1×10^6 hUCB-derived MSCs in a total 0.3 mL volume of saline was injected directly into three separate spinal cord segments (L1～L2, L2～L3, and L3～L4) using a spinal needle. Immunosuppressants were not administered to the dog. A modified Tarlove scale [13] was used to evaluate locomotor function. This scale grades hindlimb movement on a scale of 0 (no movement) to 5 (normal gait). Deep pain sensation was assessed using a hemostat to pinch the digit.

The owner of the dog reported that manual bladder expression was not needed 3 weeks after transplantation and the dog voluntarily urinated at a given location at 4 weeks during follow-up. The dog exhibited gradual improvements in hindlimb locomotor functions starting at 4 weeks post-transplantation. Modified Tarlov scores increased from 0 to 4 by 10 weeks post-transplantation. One year after transplantation, modified Tarlov scores were maintained. However, gait performance further improved although deep pain sensation was not restored after transplantation. MRI (3T; Oxford Medinus, Korea) studies were performed 3 months (Fig. 2) after transplantation. Hyperintense signals were still observed in the dorsal portion of the spinal cord even 3 months after the initial MRI examination.

The presence of FCEM is confirmed by neurohistopathologic examination showing occlusion of the spinal vasculature to nucleus pulposus that causes ischemic necrosis of dependent regions in the spinal cord parenchyma [5,7]. However, neurohistopathological examination could not be performed in the present study because the dog was still alive. Therefore, we diagnosed FCEM according to clinical symptoms, MRI results based on previous studies [1,5,8,12], and observations made during surgery. The criteria of MRI findings for diagnosing FCEM include the presence of a focal, relatively sharply

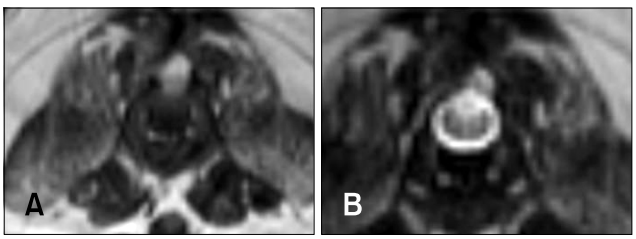

Fig. 2. T1-weighted (TR 550.0, TE 12.6) and T2-weighted (TR 4400, TE 96) MRI images of the dog 12 weeks after transplantation. The transverse T1-weighted image showed isointensity in the parenchyma of L3 (A). In the transverse T2-weighted image of L3, an area of hyperintensity was visible in the dorsal aspect of the spinal cord (B).

demarcated, and often asymmetric intramedullary lesion (edematous infracted tissue) involving gray matter with hyperintense signals on T2-weighted images and isohypointense signals on T1-weighted images [1,3].

hUCBs secrete neurotrophic factors that have been described *in vitro* as brain-derived neurotrophic factor, nerve growth factors, and neurotrophin-3 [6]. Many transplanted hUCBs can differentiate into oligodendrocytes [4]. We previously reported that hUCB-derived MSCs migrate and survive in dogs with SCI for 4 weeks [10].

Spinal cord swelling is associated with a relatively poor prognosis [7]. Corticosteroid administration was found to be helpful for reducing spinal cord swelling and minimizing secondary spinal cord injury [5]. However, no significant difference was observed with or without corticosteroid treatment in another investigation [12]. Since the dog had severe cord swelling between L1 and L3 according to the MRI findings, we performed a laminectomy to minimize secondary injury due to edematous compression. We believe, however, that while laminectomy might help minimize additional secondary damage, it is not sufficient for functional recovery.

The mechanism underlying the regenerative properties of hUCB-derived MSCs remains unclear. Nevertheless, we suggest that hUCB-derived MSCs exert potential therapeutic effects on cases of FCEM given the following findings. First, motor function of the dog improved despite the presence of poor prognostic factors including spinal cord swelling, symmetrical clinical signs, and an absence of deep pain sensation. Secondly, the recovery of motor function started at 6 weeks after onset of symptom (4 weeks after transplantation). Previous studies determined that most dogs with FCEM recover within 2 weeks after the onset of clinical symptoms while a lack of improvement within 2 weeks after disease onset is indicative of a poor prognosis [3,5,7,11,12]. This recovery pattern is similar to that reported in our previous study in which the transplantation of hUCB-derived MSCs in dogs with spinal cord injuries led to improved motor function 3 or 4 weeks post-transplantation [10].

Immunological rejection was a consideration in the present case. This is because a xenograft (human cells injected into a canine) was used without administration of immunosuppressive drugs. Nevertheless, clinical evidence of immunological rejection such as lymphocytosis, cavitation, or malasic changes on MRI was not detected.

In conclusion, our results suggest that hUCB-derived MSCs can help treat patients with FCEM who lack deep pain sensation. hUCB-derived MSCs may be a potential cell source for novel therapeutic modalities for treating different types of spinal cord infarction such as FCEM in dogs and humans.

Acknowledgments

This paper was supported by Konkuk University in 2013.

References

1. **Abramson CJ, Garosi L, Platt SR, Dennis R, McConnell JF.** Magnetic resonance imaging appearance of suspected ischemic myelopathy in dogs. Vet Radiol Ultrasound 2005, **46**, 225-229.
2. **Bagley RS.** Fundamentals of Veterinary Clinical Neurology. 1st ed. pp. 57-107, Blackwell, Iowa, 2005.
3. **Cauzinille L, Kornegay JN.** Fibrocartilaginous embolism of the spinal cord in dogs: review of 36 histologically confirmed cases and retrospective study of 26 suspected cases. J Vet Intern Med 1996, **10**, 241-245.
4. **Dasari VR, Spomar DG, Gondi CS, Sloffer CA, Saving KL, Gujrati M, Rao JS, Dinh DH.** Axonal remyelination by cord blood stem cells after spinal cord injury. J Neurotrauma 2007, **24**, 391-410.
5. **De Risio L, Platt SR.** Fibrocartilaginous embolic myelopathy in small animals. Vet Clin North Am Small Anim Pract 2010, **40**, 859-869.
6. **Fan CG, Zhang QJ, Tang FW, Han ZB, Wang GS, Han ZC.** Human umbilical cord blood cells express neurotrophic factors. Neurosci Lett 2005, **380**, 322-325.
7. **Gandini G, Cizinauskas S, Lang J, Fatzer R, Jaggy A.** Fibrocartilaginous embolism in 75 dogs: clinical findings and factors influencing the recovery rate. J Small Anim Pract 2003, **44**, 76-80.
8. **Grünenfelder FI, Weishaupt D, Green R, Steffen F.** Magnetic resonance imaging findings in spinal cord infarction in three small breed dogs. Vet Radiol Ultrasound 2005, **46**, 91-96.
9. **Hawthorne JC, Wallace LJ, Fenner WR, Waters DJ.** Fibrocartilaginous embolic myelopathy in miniature schnauzers. J Am Anim Hosp Assoc 2001, **37**, 374-383.
10. **Lee JH, Chang HS, Kang EH, Chung DJ, Choi CB, Lee JH, Hwang SH, Han H, Kim HY.** Percutaneous transplantation of human umbilical cord blood-derived multipotent stem cells in a canine model of spinal cord injury. J Neurosurg Spine 2009, **11**, 749-757.
11. **Nakamoto Y, Ozawa T, Katakabe K, Nishiya K, Mashita T, Morita Y, Yasuda N, Ishii Y, Nakaichi M, Itamoto K.** Usefulness of an early diagnosis for the favorable prognosis of fibrocartilaginous embolism diagnosed by magnetic resonance imaging in 10 small- to middle-sized dogs. Vet Res Commun 2008, **32**, 609-617.
12. **Nakamoto Y, Ozawa T, Katakabe K, Nishiya K, Yasuda N, Mashita T, Morita Y, Nakaichi M.** Fibrocartilaginous embolism of the spinal cord diagnosed by characteristic clinical findings and magnetic resonance imaging in 26 dogs. J Vet Med Sci 2009, **71**, 171-176.
13. **Tarlov IM.** Spinal cord compression studies. III. Time limits for recovery after gradual compression in dogs. AMA Arch Neurol Psychiatry 1954, **71**, 588-597.