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Case Report

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Subarachnoid transplantation of autologous neurogenically induced bone marrow derived mesenchymal stem cells for the treatment of fibrocartilaginous embolic myelopathy in two dogs

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Abstract: The objective of this study was to investigate the effects of autologous neurogenically induced bone marrow-derived mesenchymal stem cells (NIBM-MSCs) in two dogs suspected to have fibrocartilaginous embolic myelopathy (FCEM). Both dogs were paraplegic without deep pain perception (DPP) and tentatively diagnosed by MRI. Autologous NIBM-MSCs (5×10^6) were transplanted into the lumbar subarachnoid space two times with a 21-day interval for each patient. Based on a 21-month follow-up of the treated animals, the transplantation of NIBM-MSCs seems to be promising in subjects with FCEM lacking DPP.

Key words: Dog, subarachnoid transplantation, mesenchymal stem cells

1. Introduction

Fibrocartilaginous embolic myelopathy (FCEM) is a disease commonly seen in dogs that causes an acute spinal cord infarction due to occlusion of spinal vessels by incoming fibrocartilaginous material from the nucleus pulposus of the intervertebral disk in different suspected ways (1,2). Although a certain diagnosis of FCEM can be confirmed by histopathologic examination, the typical clinical presentation (peracute, nonprogressive after 24 h, without pain, and usually with asymmetric myelopathy) and the exclusion of other causes with diagnostic imaging and CSF analysis can provide antemortem diagnosis (3).

The treatment of dogs with ischemic myelopathy is still controversial. The use of corticosteroids during the acute phases has been suggested by some authors, though the beneficial effect of methylprednisolone in human patients with acute spinal cord injury is not clear. Physiotherapy is thought to have a role in stimulating neuronal plasticity and its benefits in dogs with ischemic myelopathy have been described (2). Recently the beneficial effects of different types of stem cell therapy for neural regeneration in human and veterinary medicine have gained attention. The positive effects of human umbilical cord derived mesenchymal stem cells have been reported in one dog with FCEM (4).

The aim of the present study was to report the effects of subarachnoid injections of autologous neurogenically induced bone marrow-derived mesenchymal stem cells (NIBM-MSCs) in two dogs with paraplegia secondary to FCEM (tentatively diagnosed through MRI and clinical signs) and lacking deep pain perception (DPP).

2. Case history

One of the dogs (case 1) was a 5-year-old Dachshund male intact dog with acute onset paraplegia without DPP for 2 days. The other dog (case 2) was a 2-year-old female mixed breed intact dog with acute onset paraplegia without DPP for 3 days. According to the history of both cases, the clinical signs were not progressive or symmetric.

In the neurologic examination the Texas spinal cord injury score was obtained to evaluate the neurologic score at the time of admission and to evaluate the outcomes (5). The segmental spinal reflexes and standard spinal radiographs were normal. The results of CSF analysis were unremarkable in both dogs.

The images of all cases were obtained with an MRI unit (Siemens superconducting magnet, field strength of 1.5 tesla, Siemens AG, Germany), using a spinal coil. T1 weighted images (TR: 370–700 ms, TE: 15–20 ms) and T2 weighted images (TR: 2000–4000 ms, TE: 90–110 ms) were adjusted to 2-mm slice thickness.

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Physiotherapy, including active range of motion massage to the hindlimbs and warming the thoracolumbar spine and hindlimbs, and forcing the dog to stand and walk with bellyband were recommended to the owners during the processing period of collecting bone marrow cells and also following transplantation.

Bone marrow was collected from the iliac crest and transported to the laboratory within 1 h. BM-MSCs were cultured according to standard methods (6). The cells were cultured up to passage 2 and ~5.0×10^6 BM-MSCs were separated and induced into the neurogenic lineage. The remaining BM-MSCs were cultured likewise for the subsequent booster application. BM-MSCs were induced into the neurogenic lineage by a two-step differentiation protocol (7). Immunohistochemistry revealed that these cells were positive for CNPase and MAP-2, as well as for GFAP and beta III tubulin (Abcam). The autologous NIBM-MSCs were transplanted into the L5–6 subarachnoid space two times for each case with a 21-day interval.

3. Results and discussion

In case 1, neurological examination upon admission revealed that the Texas gait score was 1 (intact limb protraction with no ground clearance), but both proprioception and nociception scores were absent (0) and the clinical signs were symmetric. Hyperintense focal unshaped and asymmetric lesions in the spinal cord parenchyma at the level of T12–L1 in T2 w images, and hypoisointense lesions in T1 w images in MRI were observed. There were also type 2 disk degenerations in the T13–L1 and L1–2 regions. The lesion length (62 mm)/L2 length (17.4 mm) ratio was 3.56 and the percent cross-sectional area was about 52.46% in T2 w images. Prior to transplantation of stem cells (42 days after admission) the neurological status was the same as it was upon admission.

In case 2, neurological examination at the time of admission revealed that the Texas walking score was the same as in case 1. A hyperintense focal asymmetric lesion in the spinal cord parenchyma at the level of T11 on T2 w FSE images, and a hypoisointense lesion in T1 w images in MRI were observed (Figures 1 and 2). No sign of disk extrusion or protrusion was seen. The lesion length (28.65 mm)/L2 length (21.1 mm) ratio was about 1.36 and the percent cross-sectional area was about 60% (4.26/7.14). Prior to transplantation of stem cells (42 days after admission) the neurologic status was as follows: gait score of 3 (intact protraction with ground clearance in more than 75% of the steps), proprioception of 1 (delayed), and nociception of 1 (deep nociception present).

The neurological status of the Dachshund 2 weeks after the second stem cell injection showed no changes. In the mixed breed dog 1 score improvement was seen in the gait and proprioception scores. At 18 months after treatment,

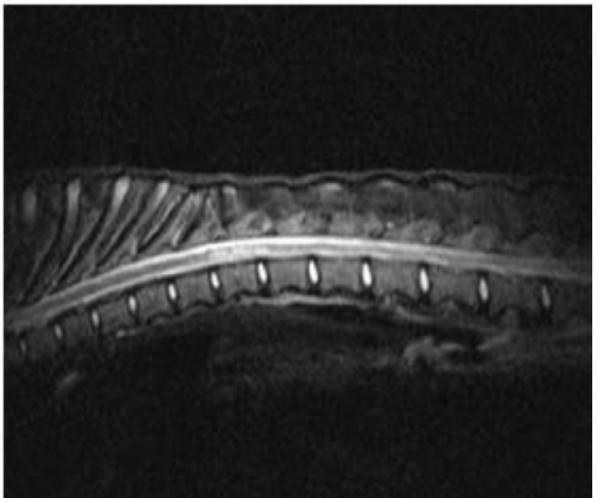

Figure 1. Hyperintense asymmetric lesion in the spinal cord parenchyma at the level of T11 on sagittal T2 w images in the mixed breed dog.

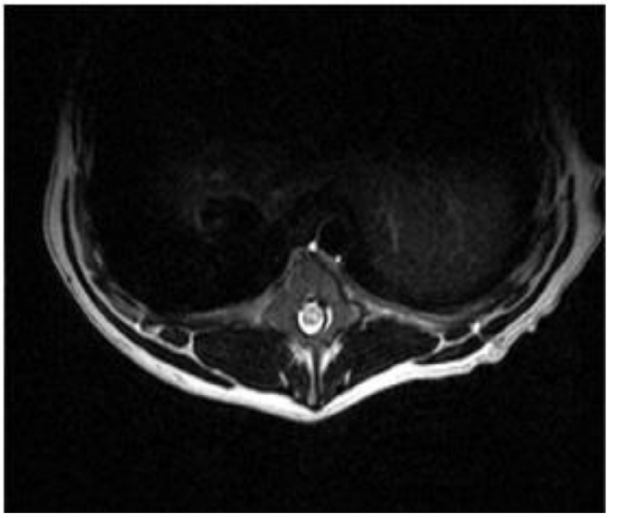

Figure 2. Hyperintense asymmetric lesion in the spinal cord parenchyma at the level of T11 on transversal T2 w images in the mixed breed dog.

the mixed breed dog had fully recovered neurologically, while the Dachshund had the same proprioception score. At 21 months after treatment, the Dachshund improved totally except for a proprioception score of 1 (delayed).

To the authors' knowledge, this is the first report of using autologous NIBM-MSCs for the treatment of FCEM in dogs. The results of the present study provide reasons for optimism in the treatment of ischemic spinal cord injury.

Characteristic MRI findings are among the most important tools in the differential diagnosis of extradural myelopathy and in defining intraparenchymal lesions (3,8). The outcome has been associated with the extent

of the ischemic intramedullary lesion on MRI, defined as the ratio between the length of the intramedullary hyperintensity on midsagittal T2-w images and the length of the vertebral body (referred to as the lesion length–L2 vertebral length ratio) and as the cross-sectional area of the largest intramedullary hyperintensity on transverse T2-w images expressed as a percentage of the cross-sectional area of the spinal cord at the same level (referred to as percent cross-sectional area of the lesion). Dogs with a lesion length–vertebral length ratio greater than 2.0, or with a cross-sectional area of the lesion (transversal images) of 67% or greater, are significantly more likely to have an unsuccessful outcome than those with lower values for these parameters. The values of our mixed breed dog were close the lower limits; however, the values of the Dachshund dog were higher. When the outcome period is considered, the results of the 2 cases were in line with a previous report (3).

The prognosis of FCEM depends on the loss of nociception, symmetric neurologic deficits, the severity of neurologic signs at initial examination quantified by a neurologic score, and the lack of spontaneous improvement within the first 14 days (1–3). The outcome has also been associated with the extent of the ischemic intramedullary lesion, and different recovery rates have been reported (3,9).

The two cases were presented with nonambulatory paraplegia without DPP, and had not been medicated before; they were subjected to cell therapy since their prognoses were evaluated as poor. While one case showed some improvement with just physical therapy on day 42 after admission, the other case did not show any improvement in the same period. The main difference between these two dogs was that the slower-improving dog was chondrodystrophic and had some type 2 disk degeneration as well. Mostly the dogs with confirmed FCEM are nonchondrodystrophic breeds and are rarely chondrodystrophic. The nucleus matrix of nonchondrodystrophic breeds remains soft and gelatinous during a longer period of time compared with that of chondrodystrophic breeds (1,10). Although both cases reported in the present study were not evaluated histologically, FCEM diagnosis was strongly suspected. The presence of type 2 disk disease without any compression complicated our tentative diagnosis, but the intraparenchymal hyperintensity and clinical signs could not be explained by disk disease alone. The unremarkable CSF results differentiated the disease from granulomatous meningoencephalomyelitis.

The treatment of dogs with ischemic myelopathy is controversial and there is no specific treatment (1,3). Recently, the clinical application of spinal cord regenerative therapy using BM-MSCs has led to promising results in human and veterinary medicine. Bone marrow stromal cells have the ability to migrate to the site of CNS injury following intravascular or intrathecal administration (11). When predifferentiated MSCs are expanded and differentiated into neurospheres in vitro, before being transplanted back, they may prove to be more beneficial for treating SCI (4). Booster transplantation of autologous NIBM-MSCs was carried out to extend the therapeutic effects of cells on the release of cytokines and on the possibility of extending the cell replacement period. The beneficial effect of autologous bone marrow-derived MSC transplantation in naturally occurring SCI was reported (12). The use of neurogenically induced allogeneic MSCs was also studied in experimentally induced spinal cord injury with promising results (13). We think that inducing autologous MSCs is more useful for SCI, except for the time needed for cell processing. However, cell therapy in the acute stage of the disease did not produce useful effects in the two cases studied.

Based on the findings of the present study, the use of autologous NIBM-MSCs seems to be a promising treatment modality for cases with guarded prognosis of FCEM embolic myelopathy in dogs. Further qualified clinical studies are needed to confirm the outcome of this treatment method.

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