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Regenerative Therapy

Original Article

Transplantation of autologous bone marrow–derived mononuclear cells into cerebrospinal fluid in a canine model of spinal cord injury

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ABSTRACT

Introduction: Spinal cord injury (SCI) is associated with severe dysfunction of nervous tissue, and repair via the transplantation of bone marrow–derived mononuclear cells (BM-MNCs) into cerebrospinal fluid yields promising results. It is essential to understand the underlying mechanisms; therefore, this study aimed to evaluate the regenerative potential of autologous BM-MNC transplantation in a canine model of acute SCI.

Methods: Six dogs were included in this study, and SCI was induced using an epidural balloon catheter between L2 and L3, particularly in the area of the anterior longitudinal ligament. BM-MNC transplantation was performed, and T2-weighted magnetic resonance imaging (MRI) was conducted at specific time points (i.e., immediately after inducing SCI and at 1, 2, and 4 weeks after inducing SCI); moreover, the expression of growth-associated protein 43 (GAP-43) was evaluated.

Results: MRI revealed that the signal intensity reduced over time in both BM-MNC–treated and control groups. However, the BM-MNC–treated group exhibited a significantly faster reduction than the control group during the early stages of SCI induction (BM-MNC–treated group: 4.82 ± 0.135 cm [day 0], 1.71 ± 0.134 cm [1 week], 1.37 ± 0.036 cm [2 weeks], 1.21 cm [4 weeks]; control group: 4.96 ± 0.211 cm [day 0], 2.49 ± 0.570 cm [1 week], 1.56 ± 0.045 cm [2 weeks], 1.32 cm [4 weeks]). During the early stages of treatment, GAP-43 was significantly expressed at the proximal end of the injured spinal cord in the BM-MSC–treated group, whereas it was scarcely expressed in the control group.

Conclusions: In SCI, transplanted BM-MNCs can activate the expression of GAP-43, which is involved in axonal elongation (an important process in spinal cord regeneration). Thus, cell therapy with BM-MNCs can provide favorable outcomes in terms of better regenerative capabilities compared with other therapies.

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1. Introduction

Spinal cord injury (SCI) leads to severe neurological dysfunction, such as motor, sensory, or autonomic paralysis. Approximately 5000 cases of SCI occur in Japan annually, which is within the estimated range of 8–246 cases per 1 million population reported in many countries [1,2]. There is currently no definitive treatment for SCI. However, various studies using experimental animal models are underway. Moreover, many attempts have been made to develop cell transplantation techniques to promote tissue

regeneration, which include the application of Schwann cells, neural stem/progenitor cells derived from fetal tissues, embryonic stem cells, induced pluripotent stem cells, olfactory ensheathing cells, mesenchymal stem cells, and bone marrow—derived mononuclear cells (BM-MNCs) [3—11].

BM-MNCs constitute a broad category of various cell types, such as stromal cells, immune cells, and mature and immature blood cells [9,10]. BM-MNCs are available immediately after isolation without the need for culturing. The use of BM-MNCs can reduce problems associated with immunological rejection, which is frequently caused by allogeneic cell transplantation [12]. Various transplantation methods, including intravenous, intramedullary, and intracerebrospinal fluid transplantation, can be used to deliver BM-MNCs to the injured spinal cord [9—11,13,14]. BM-MNCs are known to mobilize in response to injury, releasing inflammatory cytokines in the peripheral blood; these cells eventually reach the injured tissue and contribute to its regeneration [15]. Previous studies using rat models have shown that BM-MNC transplantation following SCI has a neuroprotective effect and contributes to high survival rate and functional improvement through the release of growth factors [9—11,13,14]. Functional improvement has also been reported in dogs receiving BM-MNCs [16—18]. However, the mechanisms and time course of nerve sprouting after BM-MNC transplantation following SCI in dogs remain unclear. To the best of our knowledge, no prior reports regarding BM-MNC transplantation in a canine model of SCI have been published to date, but research in this regard is considered of great importance in terms of veterinary medicine as well as future development of effective treatment strategies for human SCI. Previous human-based clinical studies have mostly assessed changes in the chronic stage of SCI, whereas the acute stage has not been well investigated. Further, assessment of degenerative changes in nervous tissue associated with the chronic stage requires the application of appropriate regenerative therapy; therefore, the role of BM-MNCs in repair processes should be investigated. Growth-associated protein-43 (GAP-43) is a protein related to axonal regeneration localized to the growth cones and presynaptic membranes of neuritis [19—21]. GAP-43 is found in neuronal cell membranes, and its expression is associated with axonal elongation [22]. GAP-43 expression is known to be upregulated in the rat spinal cord following SCI, including compression trauma, and contributes to axonal regeneration [23]. Thus, it is reasonable to consider GAP-43 as a biomarker for axonal regeneration. However, it remains unknown whether the administration of BM-MNCs can induce GAP-43 expression.

We hypothesized that high GAP-43 expression is associated with tissue findings directly linked to nerve regeneration, causing an increase in protein expression over time. Moreover, we hypothesized a reduction in T2-weighted magnetic resonance imaging (MRI) signal intensity, wherein high values are indicative of tissue damage.

In this pilot study, we aimed to evaluate the efficacy of autologous BM-MNC transplantation into cerebrospinal fluid in a canine model of SCI based on the resulting GAP-43 expression.

2. Methods

2.1. Animal selection and experimental design

In this study, we included six adult female dogs weighing 8.2—10.5 kg. SCI was induced via epidural balloon compression. The dogs were intramuscularly anesthetized with xylazine hydrochloride at a dose of 5 mg/kg, ketamine hydrochloride at a dose of 12.5 mg/kg, and atropine sulfate at a dose of 0.25 mg per dog. Anesthesia was maintained via inhalation of 2.5 % sevoflurane

during SCI. All animal experiments in this study were performed in accordance with the Guidelines for Animal Experiments of Kyoto University (1989) as well as all relevant national and international guidelines (e.g., ARRIVE guidelines). This study was approved by the ethics committee (approval number: R-17-100).

2.2. Preparation of BM-MNCs

To prepare BM-MNCs, BM cells, including stem cells, were collected under aseptic conditions, as described previously (with some minor modifications) [16]. BM-MNCs were collected from the proximal humerus with a BM aspiration needle (16-G × 2.688 in, Angiotech Pharmaceuticals, Gainesville, FL, USA). BM-MNCs were then isolated by adding a heparin-added saline solution (1 mL of heparin sodium solution in 4 mL of physiological saline [Shimizu Pharmaceutical, Shizuoka, Japan]) to 5 mL of the collected BM cells and then subjecting the resulting mixture to density gradient centrifugation. Subsequently, 10 mL of the BM-—saline mixture was layered in 4 mL of density gradient medium (density: 1.077, Lymphoprep, Nycomed Pharma, Oslo, Norway) and was centrifuged at $450 \times g$ for 30 min. The cloud-like layer of BM-MNCs was isolated, and 10 mL of physiological saline was added, following which the solution was centrifuged at $400 \times g$ for 5 min and washed twice. Next, 1 mL of the BM-MNC solution was subjected to cell counting and cytologic examination. Trypan blue dye was used for examination, and viable BM-MNCs were counted using a standard hemacytometer. The BM-MNC transplant solution (2.0×10^8 cells/ 100 µL of physiological saline) was prepared as described previously [16].

2.3. SCI induction and BM-MNC transplantation

To induce SCI while the dogs were under general anesthesia, each dog's spinal cord was compressed using an epidural balloon catheter for 30 min [24, 25]. Following anesthetic stabilization, each dog was placed in the right lateral supine position on an operating table. An 18-G spinal needle (89-mm; TOP Corporation, Tokyo, Japan) was inserted through the lumbosacral joint into the lumbar epidural space under fluoroscopic guidance (Bransist Alexa, Shimadzu Corporation, Kyoto, Japan). A guidewire was then introduced through the needle. The spinal needle was withdrawn after fluoroscopic confirmation of the guidewire position. An introducer and dilator (7-Fr Check-Flo Performer Introducer Set; Cook Medical, Bloomington, IN, USA) were inserted into the epidural space using the guidewire, after which the dilator and guidewire were withdrawn (leaving the introducer). A balloon catheter (5-Fr Fogarty, Edwards Lifesciences Corporation, Irvine, CA, USA) was inserted through the introducer into the epidural space. The balloon catheter was then advanced under fluoroscopic guidance to a position between L2 and L3. Iohexol (Iomeron 300, Eisai Co., Ltd., Tokyo, Japan) was administered using an inflation device, and the balloon catheter was inflated to the desired amount of 1.0 mL for 30 min in the epidural space. The balloon catheter was then deflated and removed. Immediately after inducing SCI, the BM-MNC transplant solution (100 µL) was injected slowly into the subarachnoid space using a 23-G needle (25-mm) to perform cisternal puncture for 30 s (for dogs 1, 3, and 5). In the control group, saline was injected using the same procedure (for dogs 2, 4, and 6). Buprenorphine (lepetan injection [0.2 mg], Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was intramuscularly administered at a dose of 10 µg/kg to induce postoperative analgesia. For dysuria, manual expression of the bladder was performed daily, and ampicillin/cloxacillin (Viccillin-S500 injection, Meiji Seika Pharma Co., Ltd., Tokyo, Japan) was intramuscularly administered at a dose of 500 mg/day for 4 weeks.

2.4. MRI

The location and extent of each injury site were examined using MRI (MAGNETOM Symphony Sonata 1.5 T, Siemens, Erlangen, Germany). T2-weighted images with a scan thickness of 2 mm were acquired in the supine position, with high-intensity images indicating lesions of considerable neurological damage [26]. A field of view of 28 cm and pixel matrix of 512×512 were adopted for each slice. Using a human knee coil, T2-weighted images (fast spin echo, repetition time/echo time = 4000/116) were captured in the sagittal plane. Lengths of all lesions were measured using medical imaging software (OsiriX, Bernex, Switzerland, www.osirix-viewer.com). The area of each spinal cord lesion was defined as the area of the anterior longitudinal ligament that showed significantly higher intensity on T2-weighted images than the intact portions of the spinal cord (equal to the strength of the anterior longitudinal ligament). MRI was performed immediately after inducing SCI (day 0) and at 1, 2, and 4 weeks after the procedure. Two of the six dogs were sacrificed under general anesthesia at three time points (1, 2, or 4 weeks after inducing SCI) after MRI, as described below.

2.5. Neurological evaluation after SCI

After inducing SCI, the neurological condition of each dog was evaluated according to the following five categories: pain without other symptoms (grade 1), ambulatory paresis (grade 2), nonambulatory paresis (grade 3), paraplegia (grade 4), and paraplegia without nociception (grade 5) [27].

2.6. Tissue preparation and histopathological procedures

To evaluate the histopathological changes, the dogs were deeply anesthetized with ketamine hydrochloride (30-mg/kg intramuscular injection), followed by an overdose (1000 mg) of intravenous pentobarbital sodium to sacrifice the experimental models. Dogs #1 and #2, #3 and #4, and #5 and #6 were sacrificed at 1, 2, and 4 weeks after inducing SCI, respectively.

All spinal cords were harvested and perfusion fixed overnight in 10 % neutral-buffered formalin. The samples were then embedded with paraffin, sectioned at a thickness of 3 μm, and stained with hematoxylin and eosin. For immunohistochemical analysis, paraffin-embedded sections were deparaffinized and boiled in an ethylenediaminetetraacetic acid solution for 20 min. The sections were then cooled at room temperature for 30 min and washed with Tris-buffered saline and Tween 20 (TBST). After blocking with Protein Block (X0909, DAKO), Dylight 488-labeled rabbit GAP-43 polyclonal antibody (NB300-143G, Novus Biologicals, Centennial, Colorado, USA) diluted in Dako REAL (S2022, DAKO, Carpinteria, CA, USA) was added to the sections, which were then incubated overnight at 4 °C. After washing with TBST, the sections were covered with VECTASHIELD Mounting Medium with DAPI (4′,6-diamidino-2-phenylindole; H-1200, Vector Laboratories, Burlingame, California, USA) to identify nuclei. Fluorescent staining was performed, and digital images were captured under a fluorescence microscope BX50 (Olympus, Tokyo, Japan).

2.7. Statistical analysis

The lengths of high-intensity regions on T2-weighted MR images of samples treated with BM-MNCs as well as those of untreated samples (i.e., controls) were compared using a mixed effects model within and between both groups at 0, 1, 2, and 4 weeks of experiment. Statistical analyses were performed using R statistical software (version 4.1.0, Foundation for Statistical Computing, Vienna, Austria). A *p*-value <0.05 was considered to indicate statistical significance.

3. Results

3.1. Neurological evaluation after SCI

All dogs included in this study developed paraplegia without nociception (grade 5) as well as urinary dysfunction. Therefore, manual expression was conducted daily for 4 weeks after inducing SCI (Table 1).

3.2. MRI evaluation

MRI results of both BM-MNC—treated and control groups initially revealed high-intensity regions on T2-weighted images. On the day of SCI, a large number of faint signals were observed in both groups (Fig. 1a and b). The length of T2-weighted high-intensity regions was 4.82 ± 0.135 and 4.96 ± 0.211 cm in the BM-MNC—treated and control groups, respectively (Table 1). In both groups, the length of T2-weighted high-intensity regions decreased with time (Fig. 2). The BM-MNC—treated group initially showed a faster decrease in the area of abnormal signal intensity than the control group. The length of these regions in the BM-MNC—treated and control groups were 1.71 ± 0.134 and 2.49 ± 0.570 cm at 1 week after SCI induction (Fig. 1c and d) and 1.37 ± 0.036 and 1.56 ± 0.045 cm at 2 weeks after SCI induction (Fig. 1e and f), respectively. At 4 weeks after SCI induction, the length of these regions became similar between the two groups (1.21 and 1.32 cm in the BM-MNC—treated and control groups, respectively) (Fig. 1g and h).

Moreover, we conducted statistical analysis to obtain estimates and *p*-values based on a mixed effects model, which are listed in Table 2. Reduction in the measured values at each time point (1, 2, and 4 weeks after SCI induction) compared with those at day 0 in the BM-MNC—treated group were as follows: -3.106 ($p < 0.001$), -3.451 ($p < 0.001$), and -3.607 ($p < 0.001$), respectively (Table 2). Similarly, reduction in the measured values at the abovementioned time points compared with those at day 0 in the

Table 1
Summary of neurological evaluation and lesion length on MR images in BM-MNC—treated and control groups.

Dog no.	Weight (kg)	Treatment	Neurological evaluation (Grade)				Lesion length on MR images (cm)			
			Day 0	Week 1	Week 2	Week 4	Day 0	Week 1	Week 2	Week 4
1	8.2	BM-MNCs	5	5	—	—	4.904	1.808	—	—
2	10	Saline	5	5	—	—	4.732	2.992	—	—
3	9.3	BM-MNCs	5	5	5	—	4.661	1.555	1.391	—
4	9.5	Saline	5	5	5	—	5.149	1.869	1.595	—
5	10.5	BM-MNCs	5	5	5	5	4.885	1.769	1.340	1.21
6	10.2	Saline	5	5	5	5	4.996	2.604	1.531	1.32

BM-MNCs, bone marrow—derived mononuclear cells; MR, magnetic resonance.
Neurological evaluation: pain without other symptoms (grade 1), ambulatory paresis (grade 2), nonambulatory paresis (grade 3), paraplegia (grade 4), and paraplegia without nociception (grade 5).
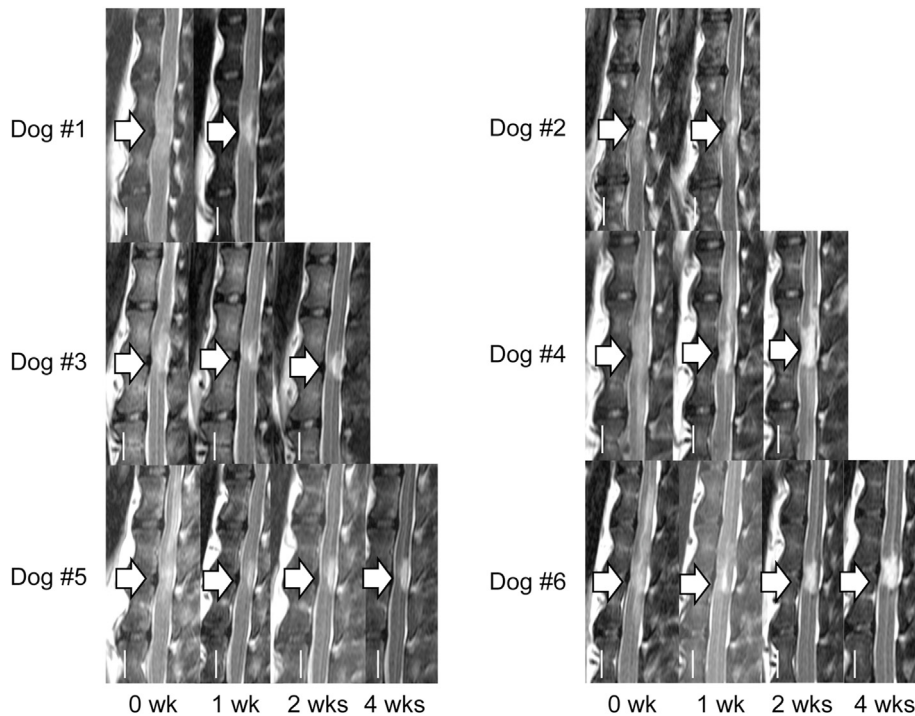
Fig. 1. Magnetic resonance imaging (MRI; T2-weighted sagittal view) of all dogs. The spinal cord samples of dogs #1, #3, and #5 were treated with bone marrow−derived mononuclear cells (BM-MNCs), and dogs #2, #4, and #6 were treated with saline. MRI was performed immediately after spinal cord injury (SCI) was induced (0 weeks) and 1, 2, and 4 weeks later. Arrows indicate contusion epicenters (white bar: 1 cm; arrow: SCI region), which were obviously increasing in size in dogs #2, #4, and #6 and remaining significantly smaller in dogs #1, #3, and #5.

control group were as follows: 0.635 ($p = 0.083$), 0.055 ($p = 0.884$), and −0.032 ($p = 0.946$), respectively (Table 2), which showed no statistically significant differences.
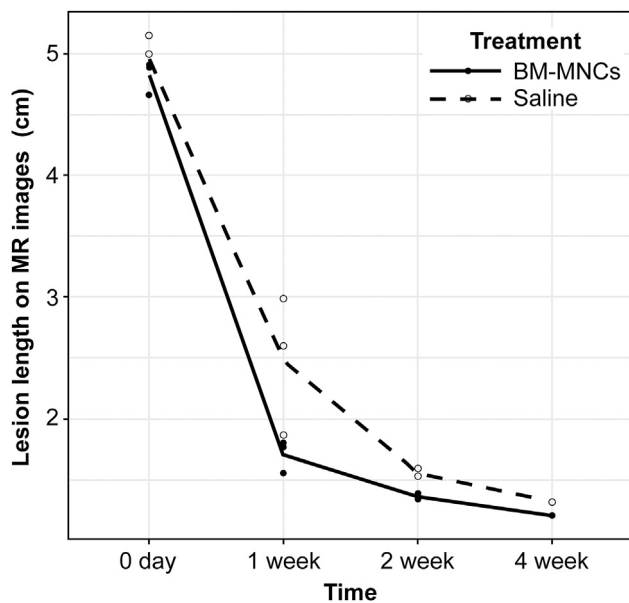
3.3. GAP-43 expression

In the BM-MNC−treated group, GAP-43 expression was clearly observed 1, 2, and 4 weeks after SCI induction (Fig. 3a). After 1 week


Fig. 2. Comparative changes in the lengths of T2-weighted high-intensity regions over time in the dogs treated with bone marrow−derived mononuclear cells (BM-MNCs) and the control group are shown on a linear graph that clearly indicates the greater reduction in length of lesion in the treated group.

of SCI induction and BM-MNC transplantation (dog #1; Fig. 4a), GAP-43 expression was detected in the spinal cord parenchyma in the vicinity of SCI. After 2 weeks of SCI induction and BM-MNC transplantation, strong fluorescence was observed in the injured spinal cord region and its limbus (dog #3; Fig. 4b). Four weeks after SCI induction and BM-MNC transplantation, continuous GAP-43 expression was noted in the limbus of SCI region (dog #5; Fig. 4c). After 1 week, in the control group, there was no GAP-43 expression; however, GAP-43 expression was moderate 2 weeks after SCI induction and obvious after 4 weeks (Fig. 3b). In the control group, we found no GAP-43 expression in the region of SCI 1 week after SCI induction (dog #2; Fig. 4d), and GAP-43 expression was barely noticeable after 2 weeks (dog #4; Fig. 4e); however, GAP-43 was significantly expressed in the spinal cord parenchyma near SCI after 4 weeks (dog #6; Fig. 4f).

4. Discussion

To the best of our knowledge, this is the first report evaluating GAP-43 expression and MRI changes following BM-MNC transplantation in SCI models. In this study, the BM-MNC−treated group exhibited more dynamic repair, as demonstrated by the faster

Table 2
A mixed effects model of lesion lengths obtained using MR images.

| Term	Estimate	p-value
Intercept	4.817	<0.001
Control (saline)	0.142	0.555
BM-MNCs (1 week)	−3.106	<0.001
BM-MNCs (2 weeks)	−3.451	<0.001
BM-MNCs (4 weeks)	−3.607	<0.001
Control 1 (1 week)	0.635	0.083
Control 2 (2 weeks)	0.055	0.884
Control 3 (4 weeks)	−0.032	0.946

BM-MNCs, bone marrow−derived mononuclear cells; MR, magnetic resonance.
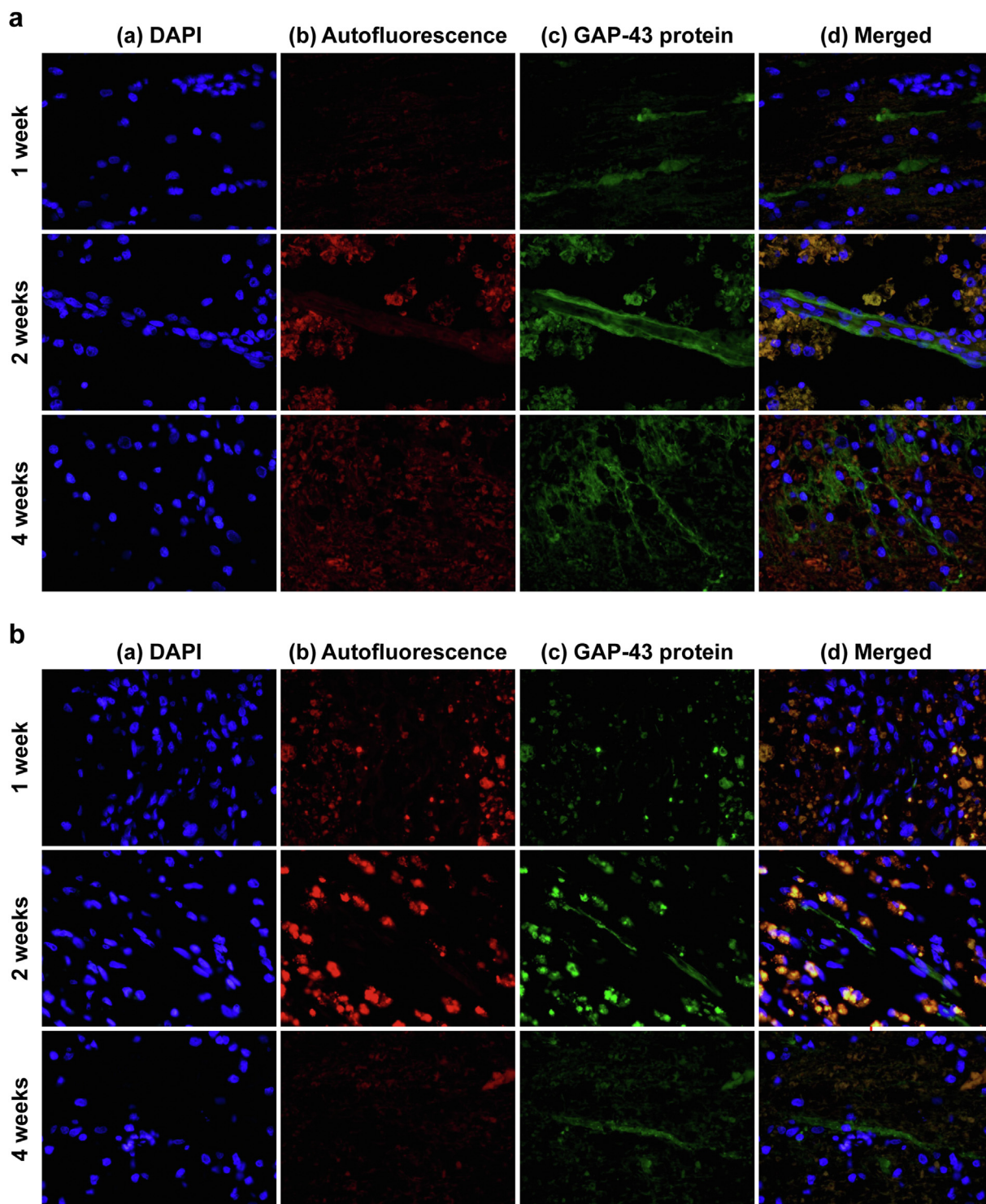
Fig. 3. Growth-associated protein 43 (GAP-43) expression following bone marrow—derived mononuclear cell (BM-MNC) transplantation in the canine model of spinal cord injury detected by fluorescence: (a) 4′,6-diamidino-2-phenylindole (DAPI). (b) Autofluorescence. (c) GAP-43 protein. (d) A merged image (×40). The BM-MNC—treated group at 1, 2, and 4 weeks (a). The control group at 1, 2, and 4 weeks (b). In the BM-MNC—treated group, GAP-43 expression was clearly observed 1, 2, and 4 weeks after SCI induction. In the control group, we found no GAP-43 expression 1 week after SCI induction; however, we detected moderate GAP-43 expression after 2 weeks and clear GAP-43 expression after 4 weeks.

reductions in length at given time points (Table 1). Compared with the control group, the negative estimates of the length of lesions (Table 2) and extremely small p-values (<0.001) in the mixed effects model in the BM-MNC—treated group indicate the negative trend in the change of the length of lesions. Therefore, lesion healing was significantly enhanced by the introduction of BM-MNC therapy. The p-value obtained through the comparison of lesion sizes between the BM-MNC—treated and control groups at 1 week after SCI induction was 0.083, which was greater than the level of significance, indicating that it is only marginally significant. However, considering the small amount of data, this finding represents a clear trend.
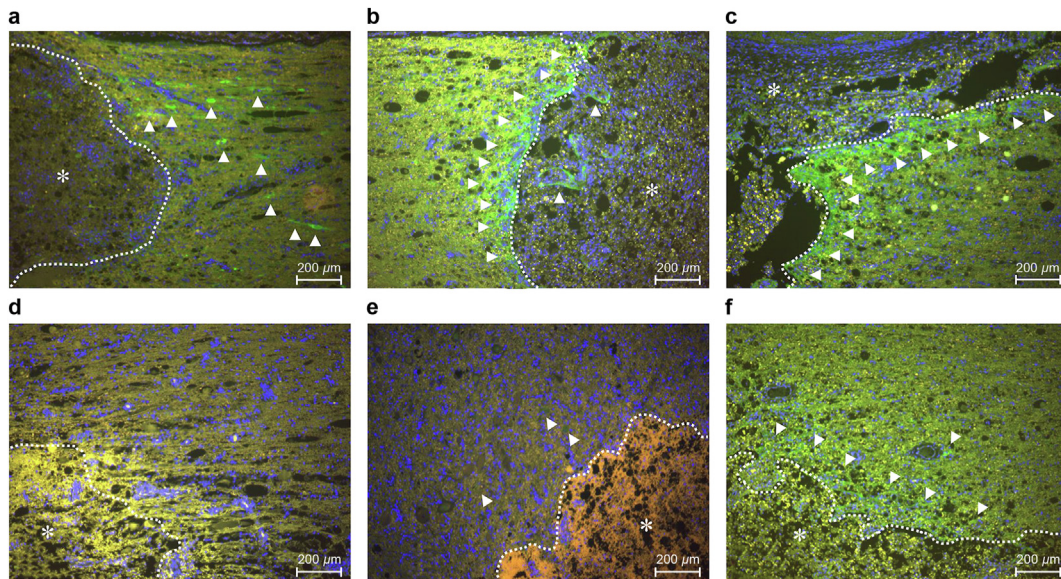
Fig. 4. Coronal sections of the spinal cord obtained from the dogs treated with bone marrow—derived mononuclear cells (BM-MNCs) (a) 1 week (dog #1), (b) 2 weeks (dog #3), and (c) 4 weeks (dog #5) after spinal cord injury (SCI) induction and BM-MNC transplantation and from the control group (d) 1 week (dog #2), (e) 2 weeks (dog #4), and (f) 4 weeks (dog #6) after SCI induction and saline treatment (bar: 200 μm). The white dotted line indicates the border between the intact and injured areas of the spinal cord (*: region of SCI). Arrowheads indicate the regions of GAP-43 expression. Merged images depict the areas with different types of fluorescence shown in specific colors (blue: 4′,6-diamidino-2-phenylindole; green: GAP-43 protein; mixed colors: autofluorescent tissues). In the BM-MSC—treated group, GAP-43 expression was sporadically observed in the spinal cord parenchyma in the vicinity of SCI 1 week after SCI induction (a). GAP-43 expression was concentrated at the border between the injured and intact areas of the spinal cord 2 weeks after SCI induction (b) and persisted in the limbus of the region of SCI 4 weeks after SCI induction (c). In the control group, we found no GAP-43 expression 1 week after SCI induction (d) and unremarkable GAP-43 expression 2 weeks after SCI induction (e). However, 4 weeks after SCI induction, we observed GAP-43 expression in the spinal cord parenchyma in the vicinity of SCI (f).

In heterogenous populations of BM-MNCs, transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor-A (VEGF-A), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and interleukin-10 are highly expressed [28]. These cytokines have been predicted to induce proinflammatory response and tissue regeneration [22—28,33]. TGF-β1 is an inducer of fibroblasts, and VEGF-A plays a crucial role in angiogenesis [28,29]. IL-10 primarily elicits an anti-inflammatory response by inhibiting inflammatory cell differentiation and secretion of inflammatory cytokines [32]. EGF promotes mitosis and activates cell division [31,33]. HGF is expected to be effective in the treatment of various diseases. Moreover, it possesses antiapoptotic properties in cells and regenerative and protective properties in various tissues [30,34—36]. It also has antifibrotic and anti-inflammatory properties [37]. IL-1b and TNF-α are known inflammatory cytokines, whose expressions are markedly downregulated in BM-MNCs [38]. These results indicate that treatment with BM-MNCs effectively suppresses the signal intensity in the early stage following SCI induction and may prevent secondary damage during SCI, as indicated by the significantly more negative estimates of lesion size in the BM-MNC—treated group at week 1. However, other cytokines such as IL-1b and TNF-α are highly expressed in BM-MNCs [39,40]. Thus, cell transplantation may lead to a temporary exacerbation of inflammation.

GAP-43 was markedly expressed in the BM-MNC—treated group, whereas it was scarcely expressed in the control group. Nerve growth factor (NGF), an important functional protein involved in numerous biological processes (including the regulation of growth, maintenance, proliferation, and survival of nerve cells), also induces GAP-43 expression [20,21]. IL-6 is involved in GAP-43 activation [38]. NGF and IL-6 are expressed in BM-MNCs [27]. These findings suggest that NGF and IL-6 in BM-MNCs promote GAP-43 expression. In the BM-MNC—treated group, GAP-43 was only sporadically expressed in spinal cord parenchyma at week 1 following SCI induction and BM-MSC transplantation. However, GAP-43 expression became more pronounced in the

injured spinal cord over time. GAP-43 is closely related to axonal elongation, especially axonal sprouting [41]. In particular, axonal regeneration starts from germination, and the direction of elongation is guided by the release of chemicals; however, a previous study demonstrated limited axonal regeneration by GAP-43 [42]. GAP-43 was expressed in the injured spinal cords evaluated in this study, suggesting that the administration of BM-MNCs increases the possibility of regeneration and elongation of injured axons in canine models of SCI. This may be associated with the significantly greater reductions in the length of lesions in the BM-MNC—treated group. In particular, the evaluation of GAP-43 expression in advanced stages of SCI facilitated by BM-MNC therapy is important, as it may correlate with the clinical manifestation of chronic diseases. Recent studies have demonstrated that BM-MNC transplantation is effective and safe, enhances functional recovery, and improves the quality of life of patients with SCI in the subacute and chronic stages, chronic traumatic brain injury, and chronic traumatic brachial plexus injury [43—46].

This study has several limitations. First, dogs were used as experimental animals in this study, and it was not possible to conduct more precise verification with a larger number of dogs. Therefore, it is necessary to enhance the evidence by accumulating research data on a restricted number of animals. In particular, the initial parameters of lesions were not thoroughly investigated in our study, which may interfere with the outcomes. Further, no association was observed between GAP-43 expression and changes in the length of lesions, indicating no direct role of GAP-43 in regeneration via axonal elongation. In addition, we could not statistically analyze the difference in GAP-43 expression between two groups as well as before and after the onset of therapy. Finally, no detailed characteristics of cell lines could be obtained. Therefore, in vitro studies with greater number of samples for comprehensive evaluation of parameters are warranted, thus achieving optimal efficacy and safety of BM-MNC transplantation in further clinical studies.

5. Conclusions

Autologous BM-MNC transplantation in a canine model of SCI facilitated the process of lesion repair, possibly enhancing GAP-43 expression regardless of its activation via injury. As GAP-43 is known to be involved in axonal elongation, an important process in spinal cord regeneration, high GAP-43 expression may result in a rapid cure of the lesion site; however, this finding should be verified in further investigations.

Declaration of interest

None.

Funding

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of Generative AI and AI-assisted technologies in the writing process

None.

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None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.reth.2023.10.003.

References

[1] Shingu H, Ohama M, Ikata T, Katoh S, Akatsu T. A nationwide epidemiological survey of spinal cord injuries in Japan from January 1990 to December 1992. Paraplegia 1995;33:183—8.
[2] Furlan JC, Sakakibara BM, Miller WC, Krassioukov AV. Global incidence and prevalence of traumatic spinal cord injury. Can J Neurol Sci 2013;40:456—64.
[3] Guest J, Santamaria AJ, Benavides FD. Clinical translation of autologous Schwann cell transplantation for the treatment of spinal cord injury. Curr Opin Organ Transplant 2013;18:682—9.
[4] Iwanami A, Kaneko S, Nakamura M, Kanemura Y, Mori H, Kobayashi S, et al. Transplantation of human neural stem cells for spinal cord injury in primates. J Neurosci Res 2005;80:182—90.
[5] McDonald JW, Liu XZ, Qu Y, Liu S, Mickey SK, Turetsky D, et al. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat Med 1999;5:1410—2.
[6] Tsuji O, Sugai K, Yamaguchi R, Tashiro S, Nagoshi N, Kohyama J, et al. Concise review: laying the groundwork for a first-in-human study of an induced pluripotent stem cell-based intervention for spinal cord injury. Stem Cell 2019;37:6—13.
[7] Mackay-Sim A, St John JA. Olfactory ensheathing cells from the nose: clinical application in human spinal cord injuries. Exp Neurol 2011;229:174—80.
[8] Neirinckx V, Cantinieaux D, Coste C, Rogister B, Franzen R, Wislet-Gendebien S. Concise review: spinal cord injuries: how could adult mesenchymal and neural crest stem cells take up the challenge? Stem Cell 2014;32:829—43.
[9] Yoshihara T, Ohta M, Itokazu Y, Matsumoto N, Dezawa M, Suzuki Y, et al. Neuroprotective effect of bone marrow-derived mononuclear cells promoting functional recovery from spinal cord injury. J Neurotrauma 2007;24:1026—36.
[10] Guo X, Bu X, Li Z, Yan Z, Jiang J, Zhou Z. Comparison of autologous bone marrow mononuclear cells transplantation and mobilization by granulocyte colony-stimulating factor in experimental spinal injury. Int J Neurosci 2012;122:723—33.

[11] Arai K, Harada Y, Tomiyama H, Michishita M, Kanno N, Yogo T, et al. Evaluation of the survival of bone marrow-derived mononuclear cells and the growth factors produced upon intramedullary transplantation in rat models of acute spinal cord injury. Res Vet Sci 2016;107:88—94.
[12] Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. Nature 2004;432:324—31.
[13] Akiyama Y, Radtke C, Honmou O, Kocsis JD. Remyelination of the spinal cord following intravenous delivery of bone marrow cells. Glia 2002;39:229—36.
[14] Samdani AF, Paul C, Betz RR, Fischer I, Neuhuber B. Transplantation of human marrow stromal cells and mono-nuclear bone marrow cells into the injured spinal cord: a comparative study. Spine (Phila Pa 1976) 2009;34:2605—12.
[15] Henrich D, Seebach C, Verboket R, Schaible A, Marzi I, Bonig H. The osteoinductive activity of bone-marrow-derived mononuclear cells resides within the CD14+ population and is independent of the CD34+ population. Eur Cell Mater 2018;35:165—77.
[16] Tamura K, Harada Y, Nagashima N, Itoi T, Ishino H, Yogo T, et al. Autotransplanting of bone marrow-derived mononuclear cells for complete cases of canine paraplegia and loss of pain perception, secondary to intervertebral disc herniation. Exp Clin Transplant 2012;10:263—72.
[17] Tamura K, Harada Y, Kunimi M, Takemitsu H, Hara Y, Nakamura T, et al. Autologous bone marrow mononuclear cell transplant and surgical decompression in a dog with chronic spinal cord injury. Exp Clin Transplant 2015;13:100—5.
[18] Tamura K, Maeta N. Efficacy of autologous bone marrow mononuclear cell transplantation in dogs with chronic spinal cord injury. Open Vet J 2020;10:206—15.
[19] Denny JB. Molecular mechanisms, biological actions, and neuropharmacology of the growth-associated protein GAP-43. Curr Neuropharmacol 2006;4:293—304.
[20] Grasselli G, Strata P. Structural plasticity of climbing fibers and the growth-associated protein GAP-43. Front Neural Circ 2013;7:25.
[21] Liu X, Cui X, Guan G, Dong Y, Zhang Z. MicroRNA-192-5p is involved in nerve repair in rats with peripheral nerve injury by regulating XIAP. Cell Cycle 2020;19:326—38.
[22] Kinnaird T, Stabile E, Burnett MS, Epstein SE. Bone-marrow-derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. Circ Res 2004;95:354—63.
[23] Curtis R, Green D, Lindsay RM, Wilkin GP. Up-regulation of GAP-43 and growth of axons in rat spinal cord after compression injury. J Neurocytol 1993;22:51—64.
[24] Nakamoto Y, Tsujimoto G, Ikemoto A, Omori K, Nakamura T. Pathological changes within two weeks following spinal cord injury in a canine model. Eur Spine J 2021;30:3107—14.
[25] Lee JH, Choi CB, Chung DJ, Kang EH, Chang HS, Hwang SH, et al. Development of an improved canine model of percutaneous spinal cord compression injury by balloon catheter. J Neurosci Methods 2008;167:310—6.
[26] Schroeder GD, Hjelm N, Vaccaro AR, Weinstein MS, Kepler CK. The effect of increased T2 signal intensity in the spinal cord on the injury severity and early neurological recovery in patients with central cord syndrome. J Neurosurg Spine 2016;24:792—6.
[27] Schulz KS, Walker M, Moon M, Waldron D, Slater M, McDonald DE. Correlation of clinical, radiographic, and surgical localization of intervertebral disc extrusion in small-breed dogs: a prospective study of 50 cases. Vet Surg 1998;27:105—11.
[28] Maeta N, Tamura K, Ezuka F, Takemitsu H. Comparative analysis of canine mesenchymal stem cells and bone marrow-derived mononuclear cells. Vet World 2021;14:1028—37.
[29] Xu F, Liu Y, Shi L, Cai H, Liu W, Hu Y, et al. RGS3 inhibits TGF-β1/Smad signalling in adventitial fibroblasts. Cell Biochem Funct 2017;35:334—8.
[30] Zheng L, Zhao C, Du Y, Lin X, Jiang Y, Lee C, et al. PDGF-CC underlies resistance to VEGF-A inhibition and combinatorial targeting of both suppresses pathological angiogenesis more efficiently. Oncotarget 2016;7:77902—15.
[31] Zhu XY, Zhang XZ, Xu L, Zhong XY, Ding Q, Chen YX. Transplantation of adipose-derived stem cells overexpressing hHGF into cardiac tissue. Biochem Biophys Res Commun 2009;379:1084—90.
[32] Tamama K, Fan VH, Griffith LG, Blair HC, Wells A. Epidermal growth factor as a candidate for ex vivo expansion of bone marrow-derived mesenchymal stem cells. Stem Cell 2006;24:686—95.
[33] Franchimont D, Martens H, Hagelstein MT, Louis E, Dewe W, Chrousos GP, et al. Tumor necrosis factor alpha decreases, and interleukin-10 increases, the sensitivity of human monocytes to dexamethasone: potential regulation of the glucocorticoid receptor. J Clin Endocrinol Metab 1999;84:2834—9.
[34] Sassone-Corsi P, Mizzen CA, Cheung P, Crosio C, Monaco L, Jacquot S, et al. Requirement of Rsk-2 for epidermal growth factor-activated phosphorylation of histone H3. Science 1999;285:886—91.
[35] Motoi S, Toyoda H, Obara T, Ohta E, Arita Y, Negishi K, et al. Anti-apoptotic effects of recombinant human hepatocyte growth factor on hepatocytes were associated with intrahepatic hemorrhage suppression indicated by the preservation of prothrombin time. Int J Mol Sci 2019;20:1821.
[36] Oliveira AG, Araújo TG, Carvalho BM, Rocha GZ, Santos A, Saad MJA. The role of hepatocyte growth factor (HGF) in insulin resistance and diabetes. Front Endocrinol 2018;9:503.
[37] Matsumoto K, Funakoshi H, Takahashi H, Sakai K. HGF-met pathway in regeneration and drug discovery. Biomedicines 2014;2:275—300.

[38] Wu X, Xu W, Feng X, He Y, Liu X, Gao Y, et al. TNF-a mediated inflammatory macrophage polarization contributes to the pathogenesis of steroid-induced osteonecrosis in mice. Int J Immunopathol Pharmacol 2015;28:351—61.

[39] Takamura H, Terashima T, Mori K, Katagi M, Okano J, Suzuki Y, et al. Bone-marrow-derived mononuclear cells relieve neuropathic pain after spinal nerve injury in mice. Mol Ther Methods Clin Dev 2020;17:657—65.

[40] Wooff Y, Man SM, Aggio-Bruce R, Natoli R, Fernando N. IL-1 family members mediate cell death, inflammation and angiogenesis in retinal degenerative diseases. Front Immunol 2019;10:1618.

[41] Kunz D, Walker G, Bedoucha M, Certa U, März-Weiss P, Dimitriades-Schmutz B, et al. Expression profiling and Ingenuity biological function analyses of interleukin-6- versus nerve growth factor-stimulated PC12 cells. BMC Genom 2009;10:90.

[42] Alibardi L. Growth associated protein 43 and neurofilament immunolabeling in the transected lumbar spinal cord of lizard indicates limited axonal regeneration. Neural Regen Res 2022;17:1034—41.

[43] Mosevitsky MI. Nerve ending "signal" proteins GAP-43, MARCKS, and BASP1. Int Rev Cytol 2005;245:245—325.

[44] Sharma A, Sane H, Gokulchandran N, Kulkarni P, Jose A, Nair V, et al. Intrathecal transplantation of autologous bone marrow mononuclear cells in patients with sub-acute and chronic spinal cord injury: an open-label study. Int J Health Sci 2020;14:24—32.

[45] Sharma AK, Sane HM, Kulkarni PP, Gokulchandran N, Biju H, Badhe PB. Autologous bone marrow mononuclear cell transplantation in patients with chronic traumatic brain injury- a clinical study. Cell Regen 2020;9:3.

[46] Sharma A, Sane H, Gokulchandran N, Badhe P, Pai S, Kulkarni P, et al. Cellular therapy for chronic traumatic brachial plexus injury. Adv Biomed Res 2018;7:51.