IJIR: Your Sexual Medicine Journal
https://doi.org/10.1038/s41443-019-0168-1

ARTICLE

Mesenchymal stem cell therapy improves erectile dysfunction in experimental spinal cord injury

Ömercan Albayrak[1] · Tarık Emre Şener[2] · Mehmet Erşahin[3] · Suna Özbaş-Turan[4] · Ceyda Ekentok[4] · Hasan Hüseyin Tavukçu[5] · Özge Çevik[6] · Şule Çetinel[7] · Büşra Ertaş[1] · Göksel Şener[1]

Received: 31 October 2018 / Revised: 13 March 2019 / Accepted: 20 April 2019
© The Author(s), under exclusive licence to Springer Nature Limited 2019

Abstract

The aim of this study is to investigate the therapeutic potential of adipose-derived mesenchymal stem cell (AD-MSC) from brown adipose tissue on erectile dysfunction (ED) in experimentally induced spinal cord injury in rats. 24 male Wistar rats were divided into 3 groups; control, spinal cord injury (SCI) + vehicle, and SCI + AD-MSC. To induce SCI, a standard weight-drop method that induced a moderate to severe injury (100 g/cm force) at T7-T10, was used. AD-MSC (3 × 105 cells /5 µL) was applied by local transplantation into the region of injury. At the end of four-weeks, rats underwent neurological examinations and then intracavernosal and mean arterial pressures (ICP and MAP) measurements. After decapitation, spinal cord and cavernosal tissue samples were taken to analyze neuronal nitric oxide synthase (n-NOS), proto-oncogene protein c-FOS and nerve growth factor (NGF). Tissues were also examined histologically. Spinal cord injury caused decrease on NGF and n-NOS levels while c-FOS was increased. The ICP/MAP value in vehicle-treated SCI rats was found to be significantly higher than the control group. On the other hand, in SCI + AD-MSC group, all these parameters were reversed back to control levels. AD-MSC therapy may be beneficial against erectile dysfunction in experimentally induced SCI by ameliorating neuronal damage.

Introduction

The pathophysiology of spinal cord injury (SCI) is characterized by initial trauma on the specific region and secondary damage where biochemical, molecular and cellular changes occur. It is well known that SCI results in physical, social and psychological negative consequences among male patients [1–3]. One of these, erectile dysfunction (ED) is an important health concern affecting the quality of life caused by SCI.

Standard spinal cord injury related animal studies are performed with an injury impacted between T7 and T10 vertebras. Erectile dysfunction following SCI has always been a debatable subject, because an ED model caused by a spinal cord trauma affecting the sacral branches has not been established as a standard traumatic SCI-induced ED model. However, there are articles that have reported successful results with a previously studied SCI-ED model [3].

In recent years, the promising results of the application of stem cells for the treatment of SCI have been shown by studies, and experimental and clinical trials of stem cells are still in progress to demonstrate the potential option of stem cells. It has been shown that, brown adipose tissue-derived mesenchymal stem cells (AD-MSCs) are capable of transforming into chondrogenic, neurogenic, and osteogenic cell types [4]. MSCs have also been shown to be derived from

✉ Göksel Şener
gokselsener@hotmail.com

1 Marmara University, School of Pharmacy, Department of Pharmacology, Istanbul, Turkey

2 Marmara University, School of Medicine, Department of Urology, Istanbul, Turkey

3 Istanbul Medeniyet University, School of Medicine, Department of Neurosurgery, Istanbul, Turkey

4 Marmara University, School of Pharmacy, Department of Biotechnology, Istanbul, Turkey

5 Haydarpaşa Sultan Abdulhamid Han Research and Training Hospital, Department of Urology, Istanbul, Turkey

6 Adnan Menderes University, School of Medicine, Department of Biochemistry, Aydın, Turkey

7 Marmara University, School of Medicine, Department of Histology & Embryology, Istanbul, Turkey

Published online: 04 July 2019

bone marrow, adipose tissue, umbilical cord blood, and fetal tissues both in animal and human studies [5, 6]. MSCs have nevre protective properties and nerve growth factors responsible for angiogenesis, synapse formation, axonal regeneration, and neurogenesis. It has also been reported that it regulates inflammation by its ability to reduce apoptosis and cytotoxic activities [7]. It is possible that MSC can regenerate themselves and differentiate into various phenotypes: chondrocytes, adipocytes, and osteocytes [7, 8].

Because MSC are able to secrete trophic factor, they exhibit antiinflammatory, antiapoptotic, antifibrotic, and immunomodulatory properties [9]. MSCs become a therapeutic option among cellular and gene therapy, tissue engineering, and in vivo studies because of their easy isolation and no response against immunity to the host tissue [10]. There is evidence that stem cell therapy provides healing in experimental SCI models and these cells have been shown to be effective in the regeneration of the nervous system after spinal cord injury in animal studies and stem cell transplantation has been considered as an alternative treatment option [11].

Although stem cell applications has been studied for the treatment of ED caused by diabetes, aging, or bilateral cavernous nerve injury in rats [12–14], there is no study on SCI-induced ED. Accordingly, in the present study we investigated the possible potential of MSCs (AD-MSC) obtained from brown adipose tissue for the treatment of ED caused by SCI.

Methods

Animal and experimental design

Male Wistar albino rats (250–300 g) supplied by the Marmara University (MU) Experimental Animal Research Center were housed in an air-conditioned room with 12:12 h light:dark cycles. The temperature (22 ± 2 °C) and relative humidity (65–70%) were kept constant. All experimental protocols were approved by the MU Animal Care and Use Committee (16.2017.mar).

Rats were randomly divided into three groups (8 rats in each): Group 1, control group (C): rats underwent sham surgery. Group 2, vehicle group (SCI), rats underwent surgery for SCI induction and received the vehicle [phosphate buffer saline (PBS)]. Group 3, SCI + AD-MSC group: rats underwent surgery for SCI induction and 3 × 105/5 μL brown adipose MSCsAD-MSCs were transplanted into the injured spinal cord via Hamilton syringe. After the trauma inductions and surgical interventions, all the rats were kept in single cages. Bladder massage was performed to prevent urinary retention.

Neurological examinations were performed 28 days following the sham surgery or SCI induction, and rats were underwent ICP measurements. After decapitation spinal cord and corpus cavernosum tissue samples were obtained for biochemical and histopathological analyses, which were all performed in a blind fashion along with all the statistical analyses.

Isolation and culture of rat adipose tissue-derived AD-MSCs

Under anesthesia with 100 mg/kg, i.p., ketamine and 1 mg/kg, i.p., chlorpromazine, 1–2 cm^3 of adipose tissue of male rats was removed from interscapular zone. The removed adipose tissue was brown adipose tissue, which was decided upon microscopic evaluation. Tissue samples were washed extensively with phosphate-buffered saline (PBS) containing 1% penicillin/streptomycin. Then, samples were placed in a sterile Petri dish with 0.075% collagenase type I for digestion, minced using scalpels and incubated for 20 min at 37 °C. Upon this step, samples were transferred to tube and centrifuged at 600*g* for 10 min for three times. Finally, supernatant were aspirated and the cell pellet was resuspended in low-glucose Dulbecco's modified Eagle's medium (Biological Industries, BI) supplemented with 15% fetal bovine serum (Pan BioTech), 1% penicillin/streptomycin (BI) and 0.02mM L-glutamine (BI). Cells were transferred to a tissue culture plate and incubated at 37 °C, 5% CO_2.

To confirm in vitro differentiation, rat MSC functional identification kit (SC020) was used [15].

Characterization of AD-MSCs

Immunophenotypic characteristics of cultured MSCs isolated from fat tissue were examined using flow cytometry. Accordingly, CD45 (eBioscience) and CD31 (Thermo Scientific) hematopoietic antigens should be negative in the cell population (not exceeding 2% of the positive rate), but the stroma-associated antigen CD90 (Thermo Scientific) must be positive on the surface of the MSCs. The MSCs obtained from the cell culture were washed once with phosphate buffer solution (PBS (SIGMA 3813)) at 1500 rpm for 5 min. After the supernatant was discarded, the cell pellet was resuspended in 1 mL of PBS (SIGMA 3813) and cell counts were performed to determine the number of cells in μL and the number of cells should be between 200,000 and 400,000. The first unpeaked cells, the second stained IgG1 FITC/IgG2a PE (Thermo Scientific) stained cells, the third stained CD31 FITC/CD90 PE stained cells and the fourth stained CD31 FITC/CD45 PE stained cells were placed. After 20 min incubation at room temperature and in the dark, sample was analyzed by using flow cytometry. BD FACSCalibur™ (San Jose, CA, USA) was used for flow

cytometric studies. AD-MSCs were identified by using the BD FACStation™ System Software (San Jose, CA, USA).

Induction of spinal cord injury

Under anesthesia (100 mg/kg, i.p., ketamine and 1 mg/kg, i.p., chlorpromazine) and sterile conditions, a skin incision was made and paravertebral muscle dissection was performed following the T5–T12 midline and spinous processes; then, the laminar arcs were removed from T7–10. SCI was induced using the modified weight-drop model [3, 16]. The dorsal surface of the spinal cord of rats was subjected to an impact of 100 g/cm (10 g weight from a 10 cm height). The weight was composed of a stainless steel rod (3-mm diameter tip) that was rounded at the surface. A 10-cm guide tube, positioned perpendicular to the center of the spinal cord, was used to drop the rod vertically onto the spinal cord. After the incision was sutured, rats were placed in a warming chamber to maintain their body temperature at approximately 37 °C until they were completely awake.

Motor function evaluation

The motor function was evaluated four times (once per week after SCI until the end of the experiment) using the method of Gale et al. [17]. Briefly the evaluation was: no movement of hindlimbs 0; perceptible movement; visible joint movements 2; hindlimb movement but cannot support body weight 3; hindlimb movement and support body weight 4; walking with mild deficit 5; normal walking 6. All behavioral tests were conducted by a “blinded” investigator. The sequence of testing animals by a given task was randomized for the animals.

Cavernous nerve stimulation and ICP/MAP measurement

On the 28th days of the study the animals were subjected to ICP/MAP measurement under general anesthesia. MAP was recorded on a computer using Biopac Student Laboratory PRO recording software (Biopac Systems, Goleta, CA, USA) via the cannulated left internal carotid artery. ICP was measured with a 24-gauge needle transducer inside the left crus of the penis. The cavernosal nerve (CN) was isolated and stimulated with a stainless steel bipolar electrode with parallel hooks (1 mm apart) around the nerve. The stimulation parameters were: 1.5 mA, 20 Hz, pulse width 5, 35 ms delay, at 7.5 V for 60 s each. The CN was stimulated and data were recorded individually. The maximum ICP/MAP ratio was calculated by dividing the highest ICP recorded during stimulation by the corresponding MAP and is represented as a percentage [3, 18, 19].

Measurement of n-NOS, NGF, and c-FOS in spinal cord and corpus cavernosum

Neuronal nitric oxide synthase (n-NOS), nerve growth factor (NGF), and c-FOS levels were quantified according to the manufacturer’s instructions and guidelines using enzyme-linked immunosorbent assay (ELISA) kits specific to the previously mentioned rat cytokines (Bioassay Technology Laboratory, E0542Ra, E0539Ra, E0046Ra).

Histopathological analysis

For light microscopic investigations, tissues were fixed in 10% formaldehyde solution and underwent routine histological preparation and were embedded in paraffin. Paraffin tissue blocks were sectioned 5 µm thickness on a rotary microtome and mounted on glass-slides. Sections of spinal cord were stained with luxol fast blue and cresyl violet (LFB&CV); sections of corpus cavernosum were stained with hematoxylin and eosin (H&E). Histologic sections were examined under an Olympus BX51 Photomicroscope for characterization of histopathological changes.

Statistical analysis

GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA) was used to perform statistical analyses. All data were expressed as means ± SEM. Analysis of variance was used to compare groups of data followed by Tukey’s multiple comparison tests. The Mann–Whitney *U*-test was used to evaluate neurological examination scores. Values of $p < 0.05$ were considered significant.

Results

Identification of brown adipose tissue

Brown adipose tissue was used to culture MSCs. The identification process of brown adipose tissue is displayed in Fig. 1a as it shows (a) multiocular brown adipose tissue, (b) plasticity demonstrating adherence, (c) red-stained vacuoles show adipogenic differentiation, (d) blue-stained cells show calcium accumulation and thus chondrogenic differentiation.

Adipose tissue-derived MSC characterization

Adipose tissue-derived MSCs were isolated and cultured in flasks for about 7 days and thereafter it was observed that they formed monolayer confluent fibroblast-like cells. We used a flow cytometer for the characterization of our isolated cells. As expected, the hematopoietic stem cell

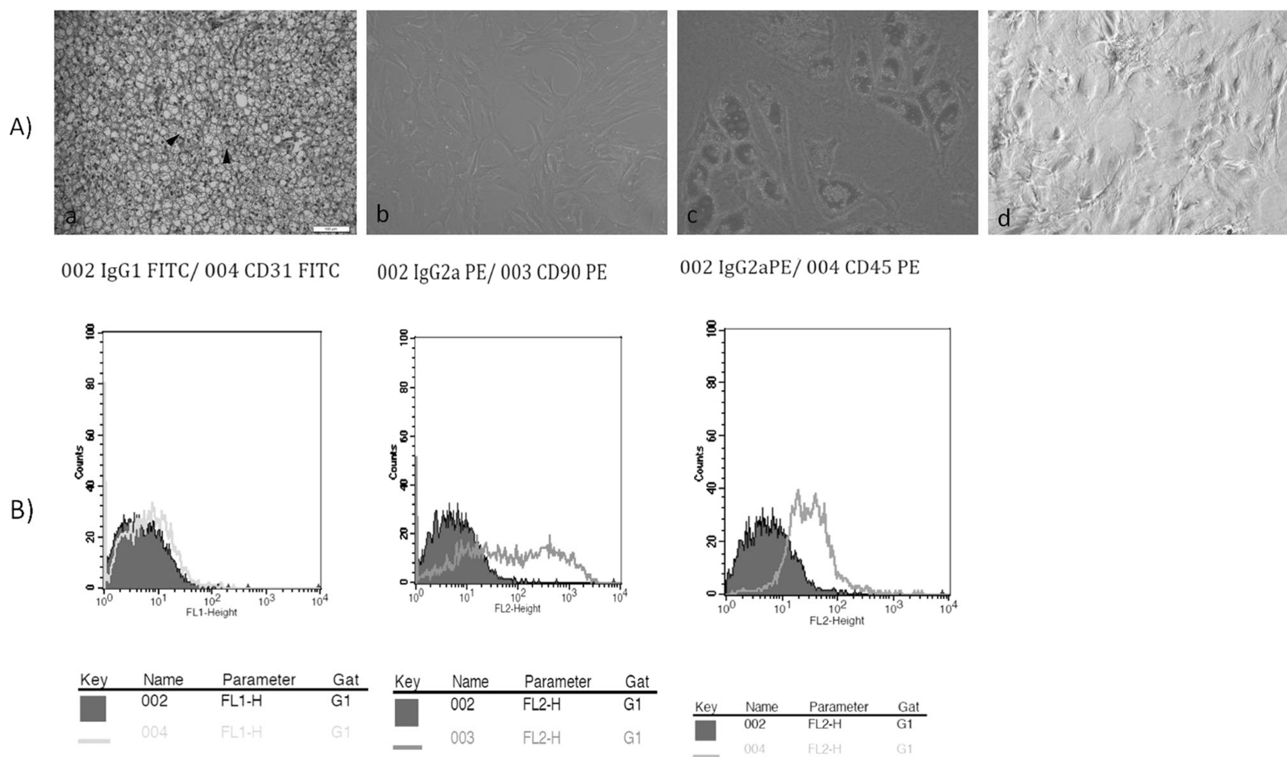


Fig. 1 a AD-MSC identification: (a) multiocular brown fat tissues (H&E staining. ×200), (b) plasticity, (c) adipogenic differentiation, and (d) chondrogenic differentiation. **b** Markers for AD-MSC characterization by using flow cytometry. Positive and negative markers, which are the result of the CD surface marker of AD-MSC

markers were negative for CD45 and CD31 and positive for stroma-associated antigen CD90 (Fig. 1b).

Motor function score

Spinal cord injury caused a significant alteration in the motor function scores (Fig. 2). According to the locomotor activity test, the SCI + AD-MSC group had higher recovery potential than the other two groups. The control and vehicle treatment group showed significantly lower activity scores than the group received stem cell (Fig. 2).

Intracavernosal pressure/MAP levels

As shown in Fig. 3, the ratio of the ICP to MAP was significantly higher in SCI + vehicle than in the control group ($p < 0.001$). On the other hand, in the SCI + AD-MSC group ICP/MAP levels were reversed back to the control group significantly ($p < 0.01$).

Neuronal NOS, NGF, and c- FOS levels in spinal cord

Both n-NOS and NGF levels of spinal cord (Fig. 4a, b) and cavernosal tissues (Fig. 4d, e) were significantly decreased ($p < 0.05$–0.01) in the vehicle-treated SCI group, while in the AD-MSC treated SCI group these values were found to be increased significantly ($p < 0.05$–0.01) and were back to the control group.

When compared to the control group, spinal cord injury did not cause a significant change in c-FOS levels for both spinal and cavernosal tissues; however, c-FOS levels of these tissues were significantly increased in the AD-MSC-treated SCI group ($p < 0.05$–0.01) (Fig. 4c, f).

Histological findings

Spinal cord tissues

Myelin damage was not encountered in spinal cord tissues of the control group (Fig. 5a). In the SCI group, staining intensity was decreased and white matter of spinal cord tissues were highly degenerated, which was suggested by intensive vacuole formation (Fig. 5b). In the SCI + AD-MSC group, spinal cord tissues had less vacuolar formation and showed nearly normal staining pattern (Fig. 5c).

Cavernosal tissues

Contrary to the normal architectural morphology of cavernosal tissues of the control group (Fig. 6a), the SCI group has moderate degenerations especially in cavernosal capillaries (Fig. 6b). The SCI group showed cork-screw shaped

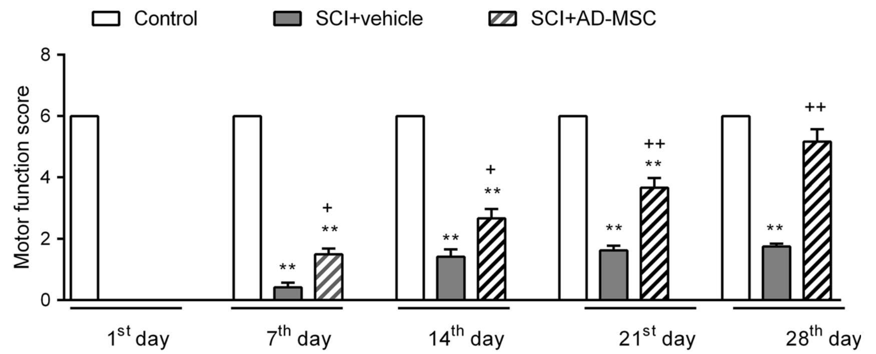
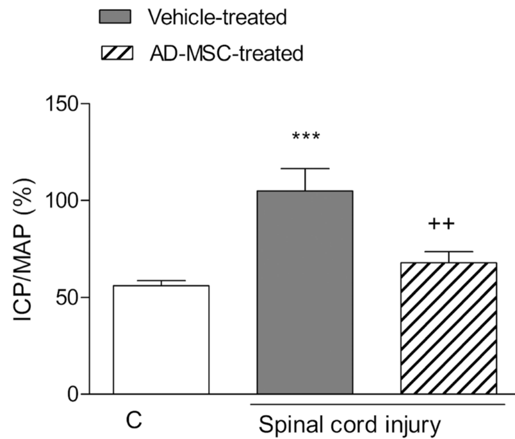


Fig. 2 Motor function score. The effect of AD-MSC therapy on paraplegia after SCI in rats. Each group consists of eight rats $^{**}p < 0.01$; vs control group, $^{+}p < 0.05$, $^{++}p < 0.01$; vs vehicle-treated SCI group. Statistical analysis was performed by Mann–Whitney *U* test



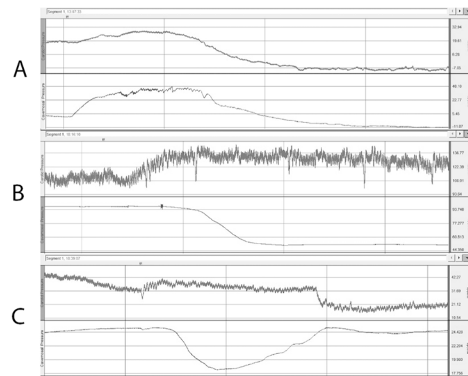


Fig. 3 The ratio of ICP/MAP for SCI groups of vehicle and stem cell treatment. Values are represented as mean ± standard error of the mean (SEM). Each group consists of eight rats. ICP intracavernosal pressure, MAP mean arterial pressure. $^{***}p < 0.001$; vs control group, $^{++}p < 0.01$; vs vehicle-treated spinal cord injury group. A: Contol, B: vehicle-treated SCI, and C: AD-MSC-treated SCI group

nuclei along with blood vessel congestion. In the SCI + AD-MSC group, congestion was less and endothelial tissues expressed normal nuclear formations with near-regular appearance (Fig. 6c).

Discussion

The data of the present study demonstrate that SCI caused oxidative tissue damage as evidenced by decreased n-NOS and NGF levels and increased in c-FOS. Furthermore, SCI caused paraplegia and severely reduced motor function scores, while another sign of damage is a significant change in erectile function. On the other hand, the findings of the present study clearly showed for the first time that treatment with AD-MSC can reverse these changes and protect cavernosal tissues against SCI-mediated tissue damage.

Since spinal cord injury is associated with permanent disability and reduced life expectancy, this traumatic event impacts a patient's physical, psychological, and social well-being and causes substantial financial burden on healthcare systems. In addition to paralysis, sensory loss and bladder/bowel dysfunction at different grades, erectile dysfunction is another important problem affecting quality of life in men [20].

Spinal cord injury needs to be evaluated as two processes; one is the primary injury induced with mechanical trauma on the impact-site and the secondary injury is involved a serious cascade of biochemical changes. The damage of neural tissue causes disruption of neural tracts and neuron loss in the spinal cord [21]. Thus, for the treatment of SCI, the target should be aimed at repairing the damaged neural tissue and eliminating complications caused by secondary events. Recently, it has been shown that stem cell treatment offers a solution for SCI treatment by providing a source of therapeutic cells for neural function restoration [22]. Accordingly in the present study we evaluated AD-MSC treatment effects on erectile function in SCI injured rats.

Nitric oxide synthase activity and cGMP levels have important effects on the NO/cGMP signaling pathway in the erectile tissue [23]. Spinal cord injury significantly reduces NOS activity and cGMP levels [3]. It is well known that NO, produced by n-NOS, is an important mediator for relaxation of cavernosal tissues and vascular smooth muscles. It has

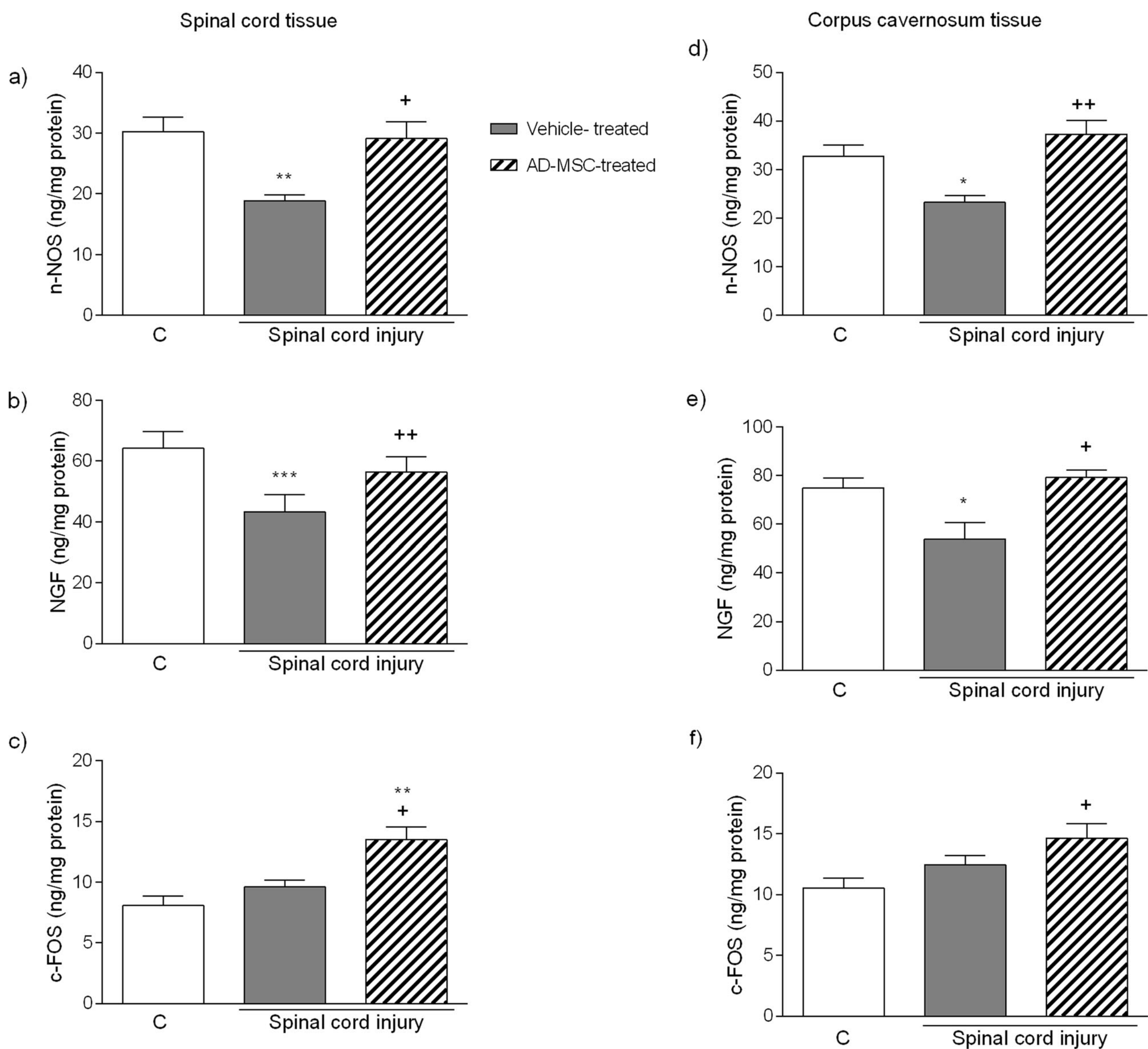


Fig. 4 n-NOS, NGF, and c-FOS results for spinal cord (**a**, **b**, **c**) and corpus cavernosum (**d**, **e**, **f**) tissues. Values are represented as mean ± standard error of the mean (SEM). Each group consists of eight rats. $*p < 0.05$; $**p < 0.01$, $***p < 0.001$; vs control group, $^{+}p < 0.05$, $^{++}p < 0.01$; vs vehicle-treated SCI group

been suggested that the reduction in n-NOS and e-NOS levels may lead to circulatory and structural changes in penile tissues, leading to ED [24]. In agreement with the aforementioned information on NO and n-NOS, in our study, SCI-induced significant reduction in n-NOS and the change was effectively reversed with AD-MSC treatment.

In similar other stem cell therapy studies, the mechanism of action of MSC therapy on SCI was still not clear. However some authors have tried to evaluate the mechanism of action of MSCs. Bucan et al. demonstrated in their study that adipose-derived mesenchymal cells secrete nanovesicles which increase neuronal growth and enhance regeneration. Also, the presence of neural growth factors transcripts has also been shown in adipose-derived MSCs' exosomes which enhance nevre regeneration [25]. Bao et al. explained in their study the mechanism of action of stem cell therapy through the types and functions of macrophages. The M1 macrophages, the proinflammatory cells, has the characteristics such as damaging nerves and inhibiting nerveregeneration in seconday inflammatory reactions; however, M2 macrophages, the antiinflammatory cells, express high levels of IL-10, TGF-B, and reduce nuclear facrot kappa B activity and up-regulate Arg1 and down-regulate proinflammatory factors. The authors stated that, MSCs, through reducing the expression of IL-7, reduced the M1 macrophage activities and also promoted the activation of M2 macrophages hence promoted repair of SCI [26].

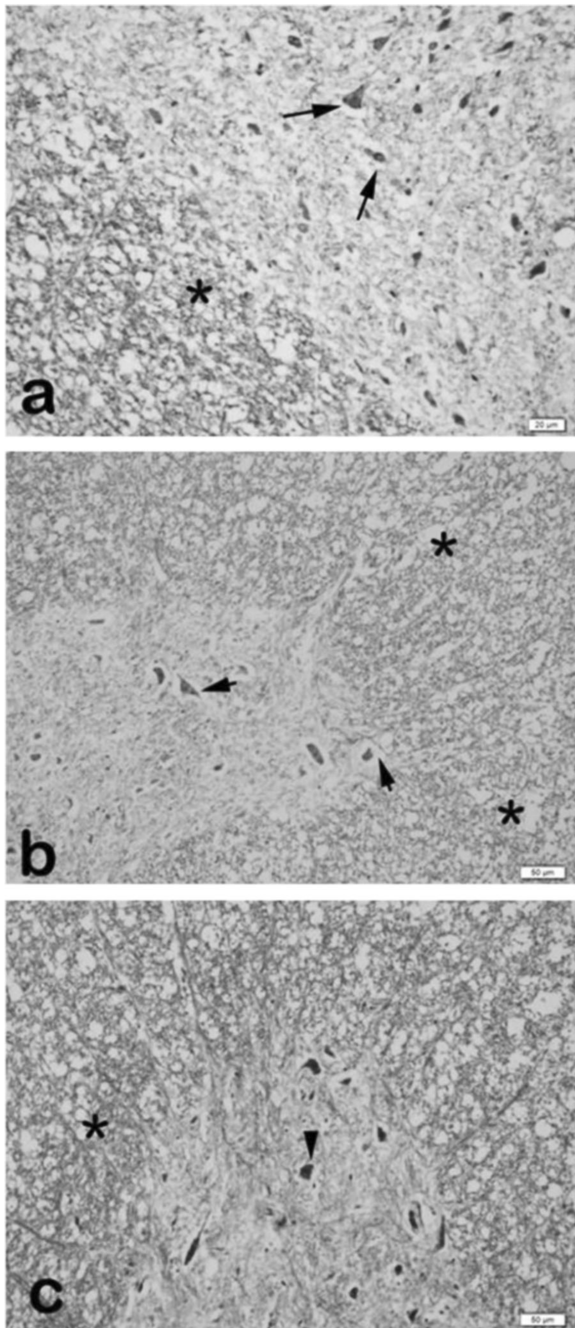


Fig. 5 LFB&CV-stained sections of spinal cords. **a** Control group: regular morphology of neurons in gray matter (arrow) and white matter (*), **b** vehicle-treated SCI group: prominent vacuole formation in white matter (*) and neurons in gray matter (arrowhead), **c** AD-MSC-treated SCI group: normal staining intensity, reduced vacuole formation in white matter (*) and reduced perineuronal space (arrowhead)

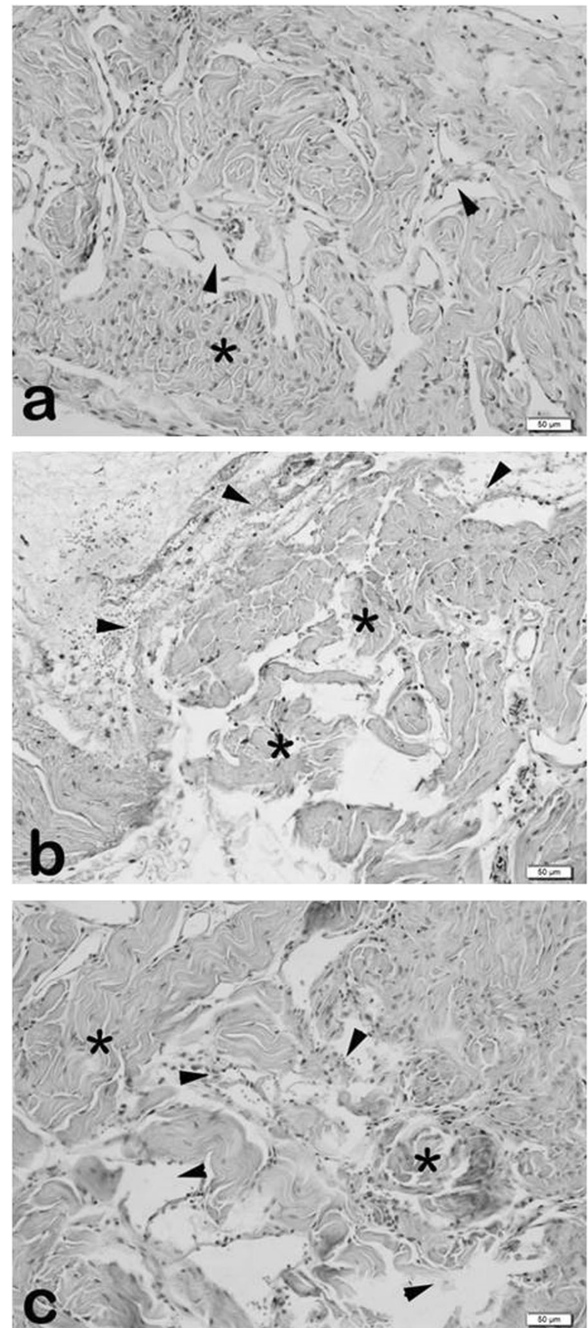


Fig. 6 H&E-stained sections of corpus cavernosum. **a** Control group: regular endothelial lining of vein (arrowhead) and collagenous, elastic, and smooth muscle fibers (*), **b** vehicle-treated SCI group: congestion in both vascular area and connective tissue (arrowhead) and disorganization in muscular tissue (*), **c** AD-MSC-treated SCI group: reduced congestion in connective tissue (arrowhead) and reorganized muscle tissue (*) ×200

ICP/MAP measurements were performed according to the technique described by Mullerad et al in their study in 2006 [18]. Although there are other described methods where different voltage measurements were also performed, Mullerad's method has been the most widely used method throughout the literature. According to Pan et al. [27], ICP measurement is the most popular technique to assess erectile function as it provides more information than apomorphine-induced erection test and is cheaper than telemetric monitoring of corpus spongiosum penis. Temeltas et al. [28] reported that ICP values measured after

cavernous nerve stimulation were higher in the SCI group than the control and the endogenous neuronal precursor cell transplanted groups. These high ICP values have not been interpreted as an erection recovery by the authors, since they reported that the penile erection was significantly weakened in the nerve stimulation test of corpus cavernosum according to the findings of sexual function analysis and evaluation of penile reflexes after spinal cord injury [28]. In our previous study, we also reported an increase in ICP/MAP values after SCI and this increase was reversed back to control group levels with subsequent treatments [3]. Similarly, in this current study, the high ICP/MAP ratio in the SCI group was reversed back to control levels with stem cell therapy. The reversal of the ICP/MAP values from those of SCI group to control levels with treatment is the indicator of erectile function recovery.

Nerve growth factor is a member of the neurotrophin family and is also important for the growth, protection, and survival of peripheral sensory and autonomic neurons [29] and also serves as a signaling molecule [30]. In our study, we observed that SCI decreased NGF levels in cavernosal tissues. In our previous study, the success of treatment against ED has been documented with an increased level of NGF in corpus cavernosum [3]. Similarly in this study, we demonstrated that, with AD-MSC treatment, the NGF level was increased and thus AD-MSC contributes significantly to the treatment of ED.

In addition, in our study, an increment of c-FOS level, a proto-oncogene, was observed in both spinal cord and corpus cavernosum tissues due to stem cell therapy in spinal cord injury. Liu et al. showed that c-FOS levels in spinal cord tissues were found to be increased after spinal cord injury [31], and as a result of MSC treatment, the expression level of the c-FOS level was increased furthermore [32]. Looking at these data, proto-oncogene c-FOS may be a potential marker of active functioning MSC presence [10].

In terms of locomotor activity tests: in the SCI group, the animals did not move their hind legs or showed a slight movement of the knee joints. The vehicle-treated SCI group with locomotor dysfunction due to spinal cord injury recovered slightly in the first 2 weeks and did not have enough hind leg movements to carry its own weight. At the end of the third week, the coordination between the anterior and posterior legs of the animals in the group treated with AD-MSC increased significantly, and hence the increase in locomotor score, and at the end of the 28th day, all the AD-MSC-treated SCI rats approached the control group levels in terms of locomotor score.

In our study, the implanted AD-MSCs have provided both biochemical and functional recovery. The erectile recovery has been observed visually along with restoration of ICP/MAP values, whereas motor function recovery has been reported with improvements in locomotor activity test scores. The possible mechanism of action is that AD-MSCs can differentiate into neuronal cells, hence restoring these aforementioned parameters. However, this differentiation has not been shown histologically, which can be sited as a limitation of our study.

Conclusion

The findings of this study demonstrate the critical pathogenic effect of reduced NGF, and n-NOS levels in the spinal cord and corpus cavernosum tissue of SCI-induced ED. Furthermore, AD-MSC therapy has an important role in SCI-induced ED since NGF and n-NOS proteins increased in both tissues after treatment. Thus, AD-MSC treatment may be possible option to prevent neurogenic ED resulting from SCI. Further studies are needed to improve efficacy of MSC.

Funding This study was supported by Marmara University Scientific Research Projects Committee with the grant number SAG-C-YLP-070317-0094.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Li X, Yang C, Li L, Xiong J, Xie L, Yang B, et al. A therapeutic strategy for spinal cord defect: human dental follicle cells combined with aligned PCL/PLGA electrospun material. Biomed Res Int. 2015;2015:197183.
2. Soler JM, Previnaire JG, Denys P, Chartier-Kastler E. Phosphodiesterase inhibitors in the treatment of erectile dysfunction in spinal cord-injured men. Spinal Cord. 2007;45:169–73.
3. Tavukcu HH, Sener TE, Tinay I, Akbal C, Ersahin M, Cevik O, et al. Melatonin and tadalafil treatment improves erectile dysfunction after spinal cord injury in rats. Clin Exp Pharm Physiol. 2014;41:309–16.
4. Lin G, Banie L, Ning H, Bella AJ, Lin CS, Lue TF. Potential of adipose-derived stem cells for treatment of erectile dysfunction. J Sex Med. 2009;6(Suppl 3):320–7.
5. Krampera M, Marconi S, Pasini A, Galie M, Rigotti G, Mosna F, et al. Induction of neural-like differentiation in human mesenchymal stem cells derived from bone marrow, fat, spleen and thymus. Bone 2007;40:382–90.
6. Xu P, Yang X. The efficacy and safety of mesenchymal stem cell transplantation for spinal cord injury patients: a meta-analysis and systematic review. Cell Transpl. 2019;28:36–46.
7. Yalvac ME, Yilmaz A, Mercan D, Aydin S, Dogan A, Arslan A, et al. Differentiation and neuro-protective properties of immortalized human tooth germ stem cells. Neurochem Res. 2011;36:2227–35.

8. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143–7.
9. Mangir N, Akbal C, Tarcan T, Simsek F, Turkeri L. Mesenchymal stem cell therapy in treatment of erectile dysfunction: autologous or allogeneic cell sources? Int J Urol. 2014;21:1280–5.
10. Karaoz E, Aksoy A, Ayhan S, Sariboyaci AE, Kaymaz F, Kasap M. Characterization of mesenchymal stem cells from rat bone marrow: ultrastructural properties, differentiation potential and immunophenotypic markers. Histochem Cell Biol. 2009;132:533–46.
11. Aras Y, Sabanci PA, Kabatas S, Duruksu G, Subasi C, Erguven M, et al. The effects of adipose tissue-derived mesenchymal stem cell transplantation during the acute and subacute phases following spinal cord injury. Turk Neurosurg. 2016;26:127–39.
12. Martínez-Salamanca JI, Zurita M, Costa C, et al. Dual strategy with oral phosphodiesterase type 5 inhibition and intracavernosal implantation of mesenchymal stem cells is superior to individual approaches in the recovery of erectile and cavernosal functions after cavernous nerve injury in rats. J Sex Med. 2016;13:1–11.
13. Yang J, Zhang Y, Zang G, Wang T, Yu Z, Wang S, et al. Adipose-derived stem cells improve erectile function partially through the secretion of IGF-1, bFGF, and VEGF in aged rats. Andrology 2018;6:498–509.
14. Zhou F, Hui Y, Xin H, Xu YD, Lei HE, Yang BC, et al. Therapeutic effects of adipose-derived stem cells-based microtissues on erectile dysfunction in streptozotocin-induced diabetic rats. Asian J Androl. 2017;19:91–97.
15. Milenkovic U, Albersen M, Castiglione F. The mechanisms and potential of stem cell therapy for penile fibrosis. Nat Rev Urol. 2019;16:79–97.
16. Allen AR. Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column: a preliminary report. JAMA. 1911;57:878–80.
17. Gale K, Kerasidis H, Wrathall JR. Spinal cord contusion in the rat: behavioral analysis of functional neurologic impairment. Exp Neurol. 1985;88:123–34.
18. Mullerad M, Donohue JF, Li PS, Scardino PT, Mulhall JP. Functional sequelae of cavernous nerve injury in the rat: is there model dependency. J Sex Med. 2006;3:77–83.
19. Sener TE, Tavukcu HH, Atasoy BM, Cevik O, Kaya OT, Cetinel S, et al. Resveratrol treatment may preserve the erectile function after radiotherapy by restoring antioxidant defence mechanisms, SIRT1 and NOS protein expressions. Int J Impot Res. 2018;30:179–88.
20. Singh A, Tetreault L, Kalsi-Ryan S, Nouri A, Fehlings MG. Global prevalence and incidence of traumatic spinal cord injury. Clin Epidemiol. 2014;6:309–31.
21. DeBrot A, Yao L. The combination of induced pluripotent stem cells and bioscaffolds holds promise for spinal cord regeneration. Neural Regen Res. 2018;13:1677–84.
22. Amemori T, Ruzicka J, Romanyuk N, Jhanwar-Uniyal M, Sykova E, Jendelova P. Comparison of intraspinal and intrathecal implantation of induced pluripotent stem cell-derived neural precursors for the treatment of spinal cord injury in rats. Stem Cell Res Ther. 2015;6:257.
23. Toksoz S, Erdem SR, Peskircioglu CL, Keskin U. The effect of long-term oral tadalafil treatment on corpus cavernosum function in an experimental spinal cord transection rat model. Spinal Cord. 2013;51:663–7.
24. Kim YW, Park SY, Kim JY, Huh JY, Jeon WS, Yoon CJ, et al. Metformin restores the penile expression of nitric oxide synthase in high-fat-fed obese rats. J Androl. 2007;28:555–60.
25. Bucan V, Vaslaitis D, Peck CT, Strauss S, Vogt PM, Radtke C. Effect of exosomes from rat adipose-derived mesenchymal stem cells on neurite outgrowth and sciatic nerve regeneration after crush injury. Mol Neurobiol. 2019;56:1812–24.
26. Bao CS, Li XL, Liu L, Wang B, Yang FB, Chen LG. Transplantation of human umbilical cord mesenchymal stem cells promotes functional recovery after spinal cord injury by blocking the expression of IL-7. Eur Rev Med Pharm Sci. 2018;22: 6436–47.
27. Pan F, Zhang J, Liu Y, Lu L, Qiu X, Lv K, et al. Intracavernosal pressure recording to evaluate erectile function in rodents. J Vis Exp. 2018;136:e56798.
28. Temeltas G, Dagci T, Evren V, Lekili M. Effects of neuronal and glial restricted precursor cells transplantation on erectile function after experimentally induced spinal cord injury. J Sex Med. 2009;6:3265–73.
29. Mnich K, Carleton L, Kavanagh E, Doyle K, Samali A, Gorman A. Nerve growth factor-mediated inhibition of apoptosis post-caspase activation is due to removal of active caspase-3 in a lysosome-dependent manner. Cell Death Dis. 2014;5:e1202.
30. Fiore M, Chaldakov GN, Aloe L. Nerve growth factor as a signaling molecule for nerve cells and also for the neuroendocrine-immune systems. Rev Neurosci. 2009;20:133–45.
31. Liu Y, Li Q, Zhang B, Ban DX, Feng SQ. Multifunctional biomimetic spinal cord: New approach to repair spinal cord injuries. World J Exp Med. 2017;7:78–83.
32. Wang L, Zhao Y, Liu Y, Akiyama K, Chen C, Qu C, et al. IFN-gamma and TNF-alpha synergistically induce mesenchymal stem cell impairment and tumorigenesis via NFkappaB signaling. Stem Cells. 2013;31:1383–95.