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Intra-bladder wall transplantation of bone marrow mesenchymal stem cells improved urinary bladder dysfunction following spinal cord injury☆

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ABSTRACT

Background: In the most of previous experiments, intrathecal administration of stem cells (SCs) was seen in the management of neurogenic bladder (NGB) following contusion or complete transaction in the rodent model of spinal cord injury (SCI). Here, we aimed to investigate whether intra bladder wall autologous bone marrow mesenchymal SC (BM-MSCs) transplantation, as a minimally invasive method, could improve bladder dysfunctions after a chronic phase of hemi- and complete-transection SCI in a female rat model.

Material and methods: A total of forty-two female Wistar rats were randomly divided into 6 groups (each in 7) and subjected to complete and incomplete spinal cord transection by a laminectomy at the T9 vertebral level. Four weeks after SCI operation, BM-MSCs (1×10^6/120 μl) were transplanted in six areas of the bladder muscle in rats with complete SCI (cSCI) and hemi SCI (hSCI) groups. In the rats from sham, cSCI and hSCI negative control groups, normal saline was injected instead of BM-MSCs. Four weeks post-cell transplantation, rats were subjected to conscious urodynamic for voiding function assessment.

Results: All bladders in cSCI and hSCI groups were the hyperreflexic type. The amplitude of uninhibited contraction in cSCI + BM-MSC group was decreased ($p = 0.046$). we noted that compliance was recovered in the hSCI + BM-MSCs group ($p = 0.041$). Residual volume was increased significantly after SCI while cell transplantation decreased this index in both hSCI and cSCI + BM-MSCs groups. The statistically significant result was only seen in the hSCI group ($p = 0.046$). Data showed that collagen deposition was markedly increased in the SCI group compared to the control or sham groups. These changes were decreased post-treatment in the hSCI group ($p = 0.042$).

Conclusion: Our study added a notion that urinary dysfunction associated with SCI, was improved following direct injection of autologous BM-MSC transplantation to bladder wall in the chronic phase of SCI injury.

1. Introduction

According to the World Health Organization reports, every year between 250,000 to 500,000 people around the world suffer from spinal cord injury (SCI), and about 11,000 new cases occur annually [2]. Following the SCI, normal motor, a sensory, or autonomic function has been changed temporary or permanent [3]. A functional, psychological and socioeconomic disorder such as neurogenic bladder (NGB), urinary tract infections (UTI), pressure ulcers, orthostatic hypotension, fractures, deep vein thrombosis, spasticity, autonomic dysreflexia, pulmonary and cardiovascular problems, and depressive disorders are frequent complications post SCI [4]. Chronic complications, especially

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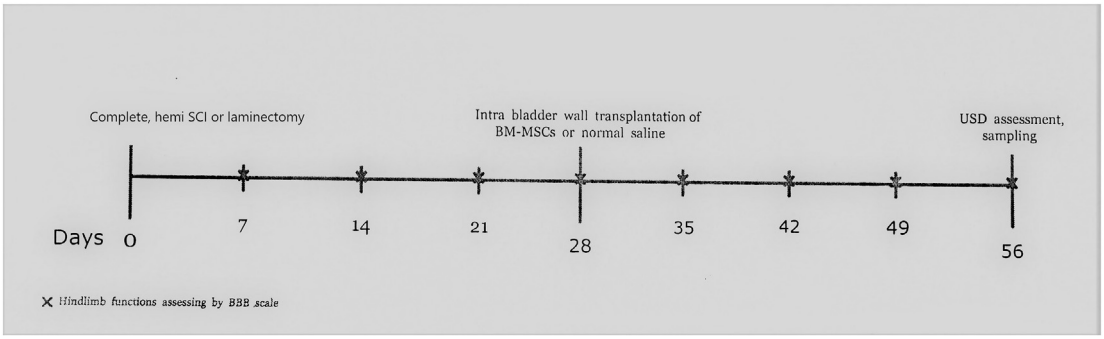

Fig. 1. Study design timeline.

urinary dysfunction and NGB, are one of the leading causes of morbidity of in these patients. In line with these descriptions, urinary dysfunction and NGB prevalence in SCI candidates seems to be between 70 and 84% [5]. Typical findings related to urodynamic study include detrusor overactivity and detrusor striated sphincter dyssynergia (DSD). These uncoordinated contractions lead to high voiding pressure, residual urine volume, and urinary incontinence that, if not treated, will contribute to renal failure [6,7]. NGB often has a significant impact on the quality of life. Incontinence has an additional negative impact on a lifestyle such as embarrassment, depression, and social isolation and if not treated optimally, may also be a risk for recurrent sepsis and pyelonephritis [8].

Therapeutic management of NGB including behavioral therapy [9], medications [10], electrostimulation [11] and surgery procedures [12] in human medicine are often accompanied with side events and/or incomplete recovery [13]. Up to the present, treatment for renal failure following the NGB disorder does not exist and thereby supportive care is the only health system efforts [14,15]. Therefore, a novel approach to recover bladder insufficiencies is highly recommended [16]. In this regard, tissue engineering and stem cell (SC) transplantation are two important options that may circumvent the inefficiencies in therapeutic systems [17]. Evidence highlight that SCs-loaded scaffolds could regenerate and restore, but not completely, the function of injured tissues via the increase of cell proliferation and induction of neovascularization rate [18].

Studies in the field of cell therapy related to bladder dysfunction are generally based on multipotent adult SCs transplantation via systemic and local administration. There are some concerns regarding SC transplantation such as ethical concerns and potential tumor formation or rejection by the recipient's immune system [19,20]. Mesenchymal SCs (MSCs) have been commonly used in the clinic for two decades. From animal models to clinical trials, MSCs showed a promise in the treatment of numerous diseases, mainly tissue injury and immune disorders [21]. Based on our knowledge, studies in the field of SC therapy for bladder dysfunction, mostly are applied to ameliorate the conditions including cancer, stress urinary incontinence, erectile dysfunction and bladder outlet obstruction (BOO) [22–26].

In the current experiment, we aimed to investigate whether intrabladder autologous bone marrow MSCs (BM-MSCs) transplantation could improve bladder dysfunctions following hemi- and complete-transaction SCI in female rats.

2. Material and methods

All of the experimental procedures were done in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institute of Health (NIH; Publication No. 85-23, revised 1985). Experiments were approved by the local ethics committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1395.24).

2.1. Animals and experimental design

A total of forty-two 13-week-old female Wistar rats (weighing 220–260 g) were enrolled in this study. Animals were housed in a 12-hour light-dark schedule with unlimited access to food and tap water. Rats were randomly divided into 6 groups (7 in each) as follows; The Control, sham-operated (sham), complete transaction SCI (cSCI) and hemisection SCI (hSCI) designated as negative control groups, cSCI group that received BM-MSCs (cSCI+ BM-MSCs) and hSCI group that received BM-MSCs (hSCI + BM-MSCs). Control rats didn't receive any intervention; sham group underwent T9–10 laminectomy by a posterior midline approach without spinal cord damage. In the cSCI group, transection was performed at T9 vertebral level with a sharp blade. Four weeks after injury, saline normal was injected into the bladder muscle. cSCI + BM-MSCs rats were received BM-MSCs; while hemisection SCI (hSCI) rats underwent on a left spinal cord hemisection at T9 level and received only saline normal was injected into bladder muscle 4 weeks after injury and, hSCI + BM-MSCs rats given BM-MSCs (Fig. 1).

2.2. BM-MSCs culture and expansion

For isolation and expansion of rat BM-MSCs, tibias and femurs were taken from 10-week-old Wistar female rats. Then, the extremities were cut and the bone marrow content flushed out by using a syringe containing 5 ml of low-glucose Dulbecco's modified Eagle medium (DMEM/LG; GIBCO). Then, an initial density of 1×10^6 cells/cm^2 were resuspended in medium with 10% fetal bovine serum (10% FBS) and 1–2% Pen-Strep and seeded in 10 cm culture dishes (SPL). After 24 h, non-adherent cells were removed by changing the medium. The medium was replenished every 2–3 days and cells allowed to reach 70–80% confluency before subculture [27–29]. BM- MSCs were used at passages 3 to 6.

2.3. Evaluation of cells prior to transplantation

Cell viability was calculated using the vital dye Trypan blue. This stain has the potential to discriminate viable cells from the dead cells [30]. For this propose, 10 µl Trypan blue solution was mixed with the same volume of cell suspension and placed in a Neubauer chamber to visualize the cell viability. To calculate the number of viable MSCs, the cells that were blue (dead) was counted.

2.4. Anesthesia and surgical procedure

Inhalational anesthesia with isoflurane was used prior to the induction of SCI and bladder catheter implantation [31]. For this propose, animals were placed in a closed box, gassed with isoflurane 5% in pure oxygen (1 L/min). After the induction of anesthesia, the drug levels were maintained at the appropriate level by a small mask with 0.8–1 L/min oxygen and 1–1.5% isoflurane.

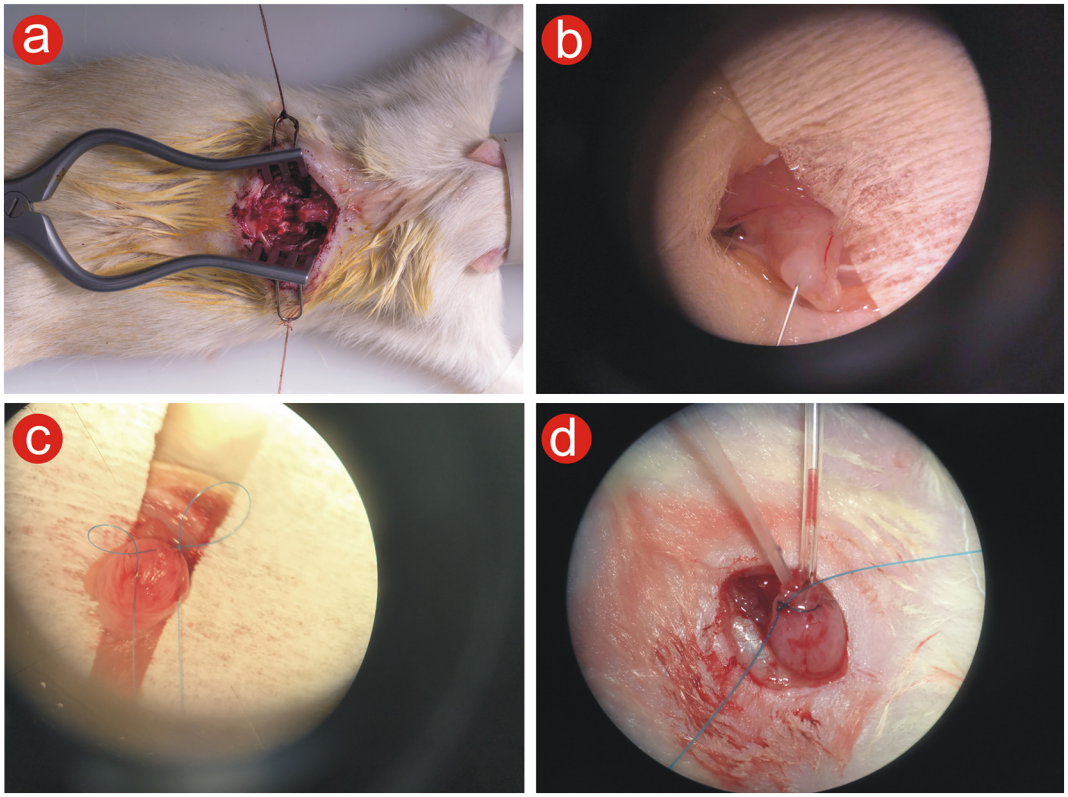

Fig. 2. Representative illustration of surgical procedure in the current experiment.
(a) Dorsal midline incision and laminectomy at the T9 vertebral level for SCI induction, (b) cell transplantation intra-bladder wall, (c) Purse-string suture in the bladder dome with 6–0 non-absorbable, monofilament suture, (d) PE 50 catheters insertion via fine incision and tightening of suture around the collar of tubes.

2.5. Induction of SCI and BM- MSCs transplantation

Anesthetized rats were subjected to complete or incomplete spinal cord transection with laminectomy at T9 vertebral level (Fig. 2). In these groups, a T9 left-sided hemisection in the rat spinal cord and complete transaction were performed with a sharp blade. The layers of skin, subcutaneous and muscle were repaired by using sutures (size 3–0). After surgical procedures, the urinary bladder was compressed twice daily for emptying. In non-responsive cases to bladder manual compression, we used polyethylene 10 (PE10) catheter via urethra for bladder evacuation. The use of urethral approach in animals was done under general anesthesia. The animals were placed in supine position, and the micturition was induced before urethra catheterization through a mild lower abdomen massage. In order to minimize the risk of infection and trauma into the urinary tract, a 10 cm long clean PE10 catheters were coated with a thin layer of medical lubricant gel.

Four weeks after SCI induction, BM-MSCs were collected using 0.25% Trypsin-EDTA solution and washed three times with PBS. For each rat in the cSCI + BM-MSCs and hSCI + BM-MSCs groups, 1×10^6 BM-MSCs were re-suspended in 120 µl normal saline and then injected carefully into six different sites at the bladder wall. To inject the cells, we used a 500-µl sterile plastic syringe (BD Syringe; Becton Dickinson and Company, Franklin Lakes, NJ, USA) (BD) connected to a 26-gauge sterile needle. In the sham group, cSCI, and hSCI groups, normal saline was injected (120 µl) into the bladder wall. The accurate intra-bladder wall injection was confirmed by the appearance of a bulge at the injection sites. Following the completion of surgery (whether for SCI or cell transplantation procedures), rats received immediately a single intraperitoneal (i.p.) injection of a 3 ml normal saline. Besides, the same rats underwent oral acetaminophen (10 mg/kg) and ciprofloxacin (1 mg/kg, i.p.) regimens for three consecutive days to reduce postoperative pain and infections, respectively.

2.6. Assessing voiding function after cells transplantation

Four weeks after cell transplantation, the rats in all study groups were subjected to conscious urodynamic assessment. Bladder catheter was inserted under anesthesia as described previously [31]. Before catheterization, PE50 tubes were heated to perform a small cuff in order to fit tightly into the bladder. For the examinations of conscious animals, the catheters were inserted through the dome of the bladder. For catheterization, the abdomen of the animal was shaved and swabbed with 70% ethanol solution and a low midline incision was done to achieve the bladder. A purse-string suture using 6–0 non-absorbable with monofilament suture was placed under a surgical microscope. After a small incision in the bladder dome, two bell-shaped tips of PE-50 catheters were inserted through a suture tightened around the collar of the tube with a surgeon's knot. Saline was infused through the catheter to ensure the lack of leakage. The other ends of the catheters were passed subcutaneously and exited through the skin by using a hollow metal rod. To check the permeability of catheters, they were flushed with saline. The analgesia was injected subcutaneously (Flunixin Meglumine, 2.5 mg/kg) and then animals were placed for 5–6 h in a restraining cage. One of the catheters was connected to an infusion pump for the continuous physiological saline infusion and the other was connected to the pressure transducer. Physiological saline was infused at room temperature into the bladder at a constant rate of 5 ml/h and intravesical pressure recorded continuously (perfusion compact, B/BRAUN Melsungen AG. Typ 8714827).

2.7. Hind limb function

We used Basso, Beattie, and Bresnahan (BBB) scale for the evaluation of the locomotor function of paralyzed hind limb after SCI. The score for lack of hind limb movement was considered as 0, and normal

hind limb movement was scored as 21 [32]. The animals from each group were examined randomly by two blinded observers after evaluation at both sides during a 4-minute time period while the rats were allowed to walk in the smooth surface open filed (cylindrical-shaped acrylic box; 90 cm diameter, 15 cm high).

2.8. Histological examination

Animals were sacrificed by Ketamine/Xylazine after urodynamic analysis [33]. Bladder tissues were taken and fixed with 4% formalin solution. Then, the tissues were paraffin embedded and 4-μm thick slides prepared. To measure the content of collagen fibers in histological slides, samples were subjected to Masson's Trichrome (MT) staining (Sigma-Aldrich). The rate of urinary tissue remodeling (collagen deposition) was analyzed using ImageJ (NIH) to estimate the areas that were stained blue. The mean blue-stained area was represented as μm^2 and compared to each other. The intensity of bladder wall fibrosis was evaluated according to the previously published studies conducted by Erdogan and colleagues as follows: Grade 0 (none) – without fibrosis and muscular hypertrophy; grade 1 (mild) – without muscular hypertrophy, only thin fibrotic tissue was observed between the epithelium and lamina propria; grade 2 (severe): muscular hypertrophy and continuous fibrotic tissue was observed among the epithelium, lamina propria, and muscularis propria [34].

2.9. Statistical analysis

Data are presented as mean ± SEM. Statistical analysis was done by using the SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA). Differences between groups were examined by One-way analysis of variance (ANOVA) and Tukey post-hoc test. $p < 0.05$ was considered significant.

3. Results

3.1. Cell viability

Based on data obtained from cell survival assay (Trypan Blue staining), we found that the viability of cell was near to 99%. These data demonstrated an appropriate viable cell number prior to cell injection to the target sites.

3.2. Mortality during and after SCI

We found a mortality rate of 30% in cSCI group and 10% in hSCI. The main causes were urinary retention that was unresponsive to bladder massage. In cSCI animals that received BM-MSCs, two rats died. We introduced additional animals to reach a minimum of 5 rats per group.

3.3. Evaluation of urination pattern

After the induction of SCI, all of the animals in cSCI groups showed urinary retention, and we found macroscopic hematuria in 21.4% (n = 3) of animals. At the end stage of this experiment, the bladder reflex didn't return to the normal function eight weeks after injury and in 4 rats of cSCI group (1 in cSCI + BM-MSCs and 3 in cSCI negative control groups) while manually emptying were necessary during the study period. In the hSCIs group, all rats showed urinary retention in the first week after injury while bladder reflex returned.

3.4. Bladder volume and urodynamic study

Urodynamic analysis showed that all bladders in cSCI and hSCI groups were the hyperreflexic type (Fig. 3). The amplitude of uninhibited contraction in cSCI group received BM-MSC showed significant differences compared to the negative control groups ($p = 0.046$). We found that compliance was recovered in rats from hSCI + BM-MSCs ($p = 0.041$). Residual volume was increased significantly after SCI and decreased in both hSCI and cSCI + BM-MSCs treatment groups after cell transplantation, but statistically significant was seen only in the hSCI group ($p = 0.046$). There was no difference in micturition frequency and baseline pressure between all groups. Based on our observations, the inter contraction interval (ICI) index was decreased which coincided with an increased peak pressure after SCI, but the value recovered post-cell transplantation. The mean bladder peak pressure was increased in both hemi- or -complete transaction rats while this value decreased after cell transplantation. However, we found non-statistically significant results (Table 1).

3.5. Body and bladder weight

The measure of body weights between the groups did not show significant differences at the first stage of the experiment ($p = 0.58$). The rats from cSCI and hSCI groups showed a higher bladder weight compared to the control or sham groups. After cell transplantation, bladder weight was lower in BM-MSCs groups in comparison with the SCI negative control groups. The reduction rate was more obvious in hSCI group ($p = 0.009$) (Fig. 4).

3.6. Hindlimb functions

Seven days after induction of hemisection SCI, the analysis by open-field locomotion BBB scale showed a mean ± SEM score of 2.62 ± 0.26. This score was improved slightly every week until 8 weeks. In rats with completely transected SCI, a BBB score of 1.14 ± 0.26 was achieved without weight support 7 days after injury. Although this score was recovered until week 8 post-injury and didn't indicate a weight support score (6.20 ± 0.37; $p = 0.75$) (Fig. 5).

3.7. Changes in collagen content

Histological examination revealed that the thickness of urothelium and lamina propria layers was higher in the injured rats following SCI compared to the control or sham groups. The distributions of bladder muscle (red stain) and fibrous tissue (blue stain) were analyzed by the MT staining. Data showed that collagen deposition increased markedly in the SCI group compared to the control or sham groups. After cells treatment, these changes were only decreased in the hSCI group ($p = 0.042$; Table 2, Figs. 6 and 7).

4. Discussion

In the current study, we induced both complete or hemi transection SCI at the level of T9 vertebrae to mimic overactive NGB. At the end of the experiment, features showed rhythmic intravesical pressure waves in the filling phase before voiding in the injured rats coincided with hyperreflexic type activity. Consistent with the results from previous experiments and this study, the ability of bladder and external urethral sphincter control were faint in animals with SCI [35,36]. The continuous contractions of the bladder were coincided with sphincter DSD, contributing to an inefficient voiding and urinary retention [37]. These changes yielded a complete deterioration of bladder compliance, function, UTI, and etc. [38]. After intrabladder cell transplantation, the basal and peak pressure in both hemi – or – complete transaction rats were decreased, but it did not contribute to statistically significant results. The amplitude of uninhibited contraction was decreased significantly in the cSCI group post cell transplantation. We also noted the improvement of residual volume (bladder capacity (BC) - micturition volume), compliance ($BC/(P_{threshold} - P_{baseline})$) [39] and bladder weight only in rats from hSCI + BM-MSCs group.

A series of animal experiments [40–46] and clinical trials [47–53]

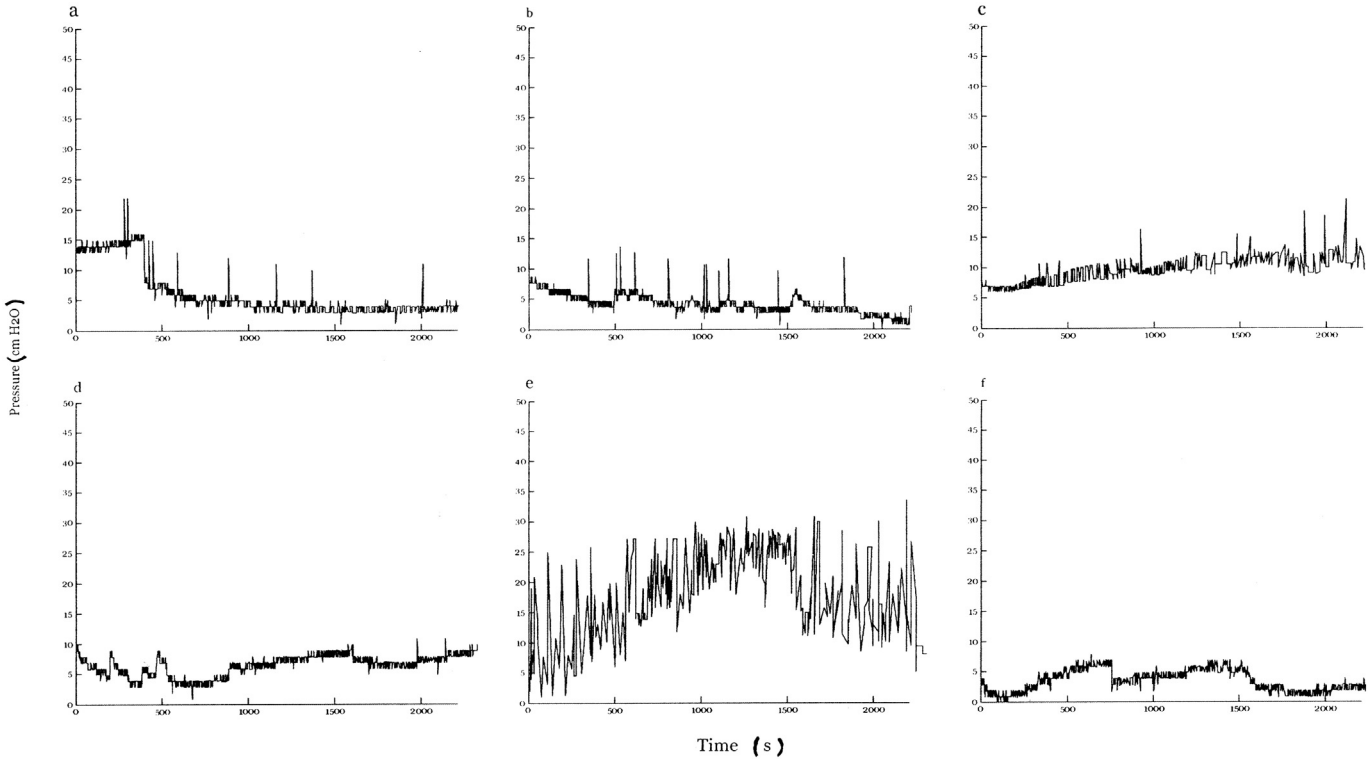

Fig. 3. Graphs of cystometry in each group. (a) Control (b) Sham (c) hSCI, (d) hSCI + BM-MSCs, (e) cSCI (f) cSCI + BM-MSCs.

Table 1
Urodynamic parameters in the control, sham-operated, hSCI and cSCI negative control groups and, hSCI and cSCI + BM-MSCs groups.

Variable/groups	Control (n = 7)	Sham (n = 7)	hSCI (n = 6)	hSCI + BMSCs (n = 6)	cSCI (n = 5)	cSCI + BMSCs (n = 5)
Peak pressure (cmH2O)	11.00 ± 1.80	16.36 ± 1.30	25.00 ± 3.28	14.25 ± 2.52	35.80 ± 10.71	19.50 ± 6.58
Baseline pressure (cmH2O)	5.16 ± 2.09	8.03 ± 1.25	15.50 ± 1.96	10.83 ± 2.09	25.23 ± 5.58	15.88 ± 6.17
Frequency (time/min)	1.21 ± 0.32	1.18 ± 0.23	0.87 ± 030	1.16 ± 0.35	0.32 ± 0.07	0.41 ± 0.12
Amplitude of uninhibited contraction* (cmH2O)	8.52 ± 1.82	9.94 ± 0.76	13.12 ± 1.72	11.08 ± 2.10	26.57 ± 5.28	14.24 ± 4.13
#ICI (Sec)	264.06 ± 23.82	163.44 ± 23.88	30.84 ± 7.30	45.04 ± 10.46	12.45 ± 2.35	26.46 ± 3.58
Compliance* (ml/cmH2o)	0.101 ± 0.008	0.088 ± 0.010	0.017 ± 0.011	0.080 ± 0.004	0.012 ± 0.004	0.039 ± 0.011
Residual volume* (ml)	0.38 ± 0.08	0.38 ± 0.06	1.80 ± 0.34	0.70 ± 0.14	2.26 ± 0.21	1.42 ± 0.18

Data are reported with mean ± SEM.
#ICI: Intercontraction interval.
* Amplitude of uninhibited contraction only in cSCI group ($p = 0.046$), compliance ($p = 0.041$) and residual volume only ($p = 0.022$) in hSCI group were improved statistically significant with cell transplantation by Tukey Post Hoc analysis. The mean difference is significant at the 0.05 level.

have previously demonstrated the therapeutic effects of SC in the SCI subjects. In an experiment conducted by Lee and co-workers, the maximum voiding pressure was decreased after B10 cell transplantation while the ICI index increased [45]. Despite the increase in ICI levels, but it did not reach to a significant level following cell transplantation. The other authors reported different urodynamic parameters. In an SCI model using a needle, Cho and et al. used oral mucosa SCs immediately at the site of injury 21 days after transplantation. They evaluated bladder basal contraction pressure and contraction time on the rats [54]. They found that only basal contraction time was improved significantly compared to the SCI negative control group. In another study directed by Park et al., the transplantation of MSC didn't recover bladder dysfunction in contrast with our data. The voiding frequency and maximum pressure didn't change [40].

In the most of previous experiments, intrathecal administration of stem cells (SCs) was seen in the management of neurogenic bladder (NGB) following contusion or complete transaction in a rodent model of SCI, but these methods have some concerns [55–57]. For instance, the intralesional injection of SCs does not contribute to remyelination. In addition, the transplanted cells could migrate into the normal tissue areas and consequently leads to further axonal damage. During the systemic injection of SCs, host immune response is provoked. Lumbar puncture delivery of MSC appears to be superior to systemic delivery into the injured spinal cord [58]. Besides, a minimally invasive method may be beneficial in MSCs transplantation to reach the best outcomes with minimum systemic side effects.

To our knowledge, there was only one study that studied the direct transplantation of human-derived-MSCs into the bladder wall and investigated bladder function following SCI in the preclinical stage [45]. Our study added a notion that urinary dysfunction associated with SCI,

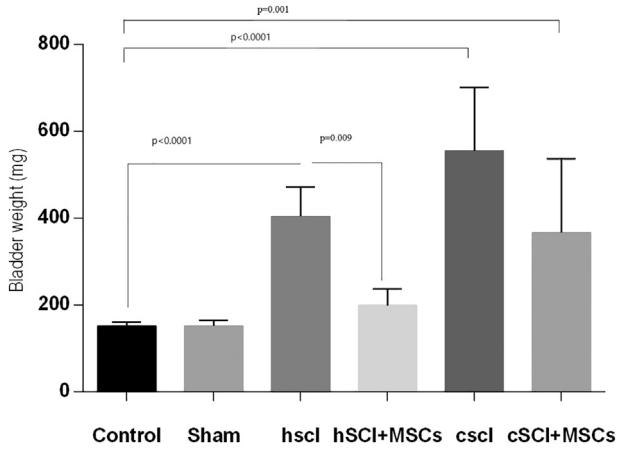

Fig. 4. The bladder weight in rats from different groups. In rats from hSCI and cSCI, an increased bladder weight was observed as compared with the control and sham-operated groups. Only the hSCI + BM-MSCs groups showed significantly decreased bladder weights compared to the hSCI group.

may improve after the transplantation of autologous BM-MSC when directly injected to the bladder wall. The precise contributory effect of BM-MSCs to restore bladder detrusor dysfunction needs to be elucidated. It was shown that BM-MSCs have potential to easily trans-differentiated into different cell types [59], such as smooth muscle cells [60,61], nerve cells [62,63] and blood vessels in the bladder under appropriate conditions [28,64,65]. BM-MSCs have the potential to prohibit any immunological rejection by secretion of several growth factors [49]. Transplantation of these cells in the subacute phase (10–14 days post-injury) is shown to be more effective than the acute phase. The acute phase is accompanied by secondary cascade events [66–68]. Based on the experiments, the therapeutic window for cell transplantation is around 7–21 days after the SCI [40,55,67,69]. However, we transplanted BM-MSCs 4 weeks after SCI in the chronic phase and found that these cells had promising outcomes in the management of NGB. Therefore, one could hypothesize that the transplantation of MSCs could contribute to the therapeutic effects during the initiation of chronic changes in the target tissues. Factors such as cell population, the timing of intervention and ability of spontaneous recovery in rodents may confound the interpretation of our results compared to the human counterpart.

The method of SCI induction, time and route of cell transplantation was different in various experiments. In addition, different SCI models such as hemisection, complete transaction, contusion, and segment

Table 2
Bladder wall fibrosis values in all groups.

Groups	Grade 0	Grade 1	Grade 2
Control (n = 10)[a]	9	1	0
Sham (n = 8)	6	2	0
hSCI (n = 10)	1	6	2
hSCI + BM-MSCs (n = 10)	5	5	0
cSCI (n = 10)	2	4	4
cSCI + BM- MSCs (n = 8)	1	5	0

*Grade 0 (none) – without fibrosis and muscular hypertrophy; grade 1 (mild) – without muscular hypertrophy, only thin fibrotic tissue was observed between the epithelium and the lamina propria; grade 2 (severe): muscular hypertrophy and continuous fibrotic tissue was observed among the epithelium, the lamina propria, and the muscularis propria.

[a] (n) represents the number of analyzed slides in each group.

resection were designed in pre-clinical settings [70–72]. It is believed that SCI transaction model is the most appropriate animal model. The pathological changes were shown to last 4 weeks post-injury and this model should be used as the standard model for the examination of SCI [71]. We found no signs in motor and/or sensory function [73]. All animals in complete SCI groups had urinary retention and approximately 30% of mortality rate was seen due to manual bladder compression for emptying urine. The previous studies used cystocentesis for bladder emptying in the non-responsive cases to bladder manual compression after SCI [74]. Here, we used the PE10 catheter via urethra for bladder evacuation in urinary retention cases. Similar to the previous data, rats died in the first week after catheterization. This shows that catheterization is not a safe method like cystocentesis.

In this study, we noted a slight improvement in the normal activity of locomotor system post-hemi or -complete transaction but no normal status was obtained even in rats subjected to cell transplantation. According to a great body of documents, SCI transection with 5% intact nerve fibers could return paraplegic hind limbs in the rat model during partial or complete injury. By reaching the residual rate to 40% or more, the locomotor function could be improved even to normal state due to the existence of a compensatory mechanism of contralateral normal nerve fibers that resulting in a gradual restoration to basic levels [75–77]. Our data are consistent with above-mentioned findings. Based on our result and previous experiments, MSCs have the potential to reduce fibrosis and collagen deposition in the hSCI model as well as the liver, lung, and bladder in BOO [45,78–80].

Despite the surprising results of intra-bladder transplantation of autologous BM-MSCs in the chronic phase of bladder dysfunction after SCI, two rats died. We couldn't find a meaningful improvement of

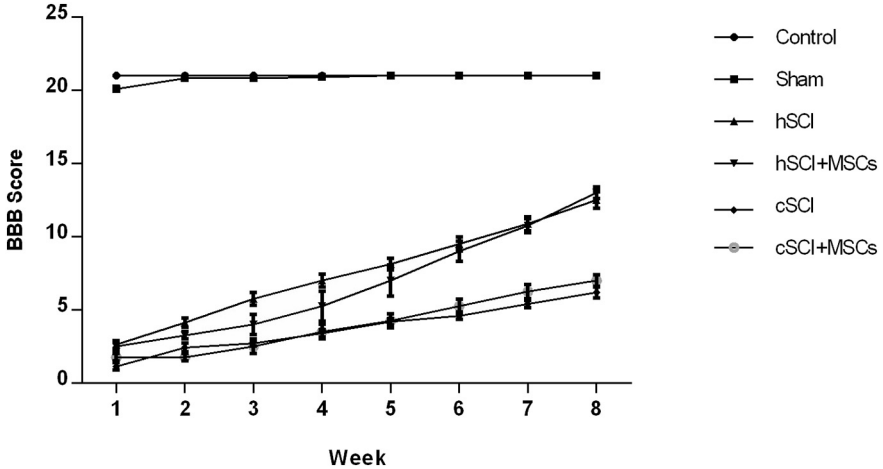

Fig. 5. Functional test of the SCI model after BM-MSCs transplantation. BBB open-field locomotor scores for the control, sham-operated, hSCI and cSCI negative control groups and, hSCI and cSCI + BM-MSCs groups tested every week till 4 weeks after cell transplantation.

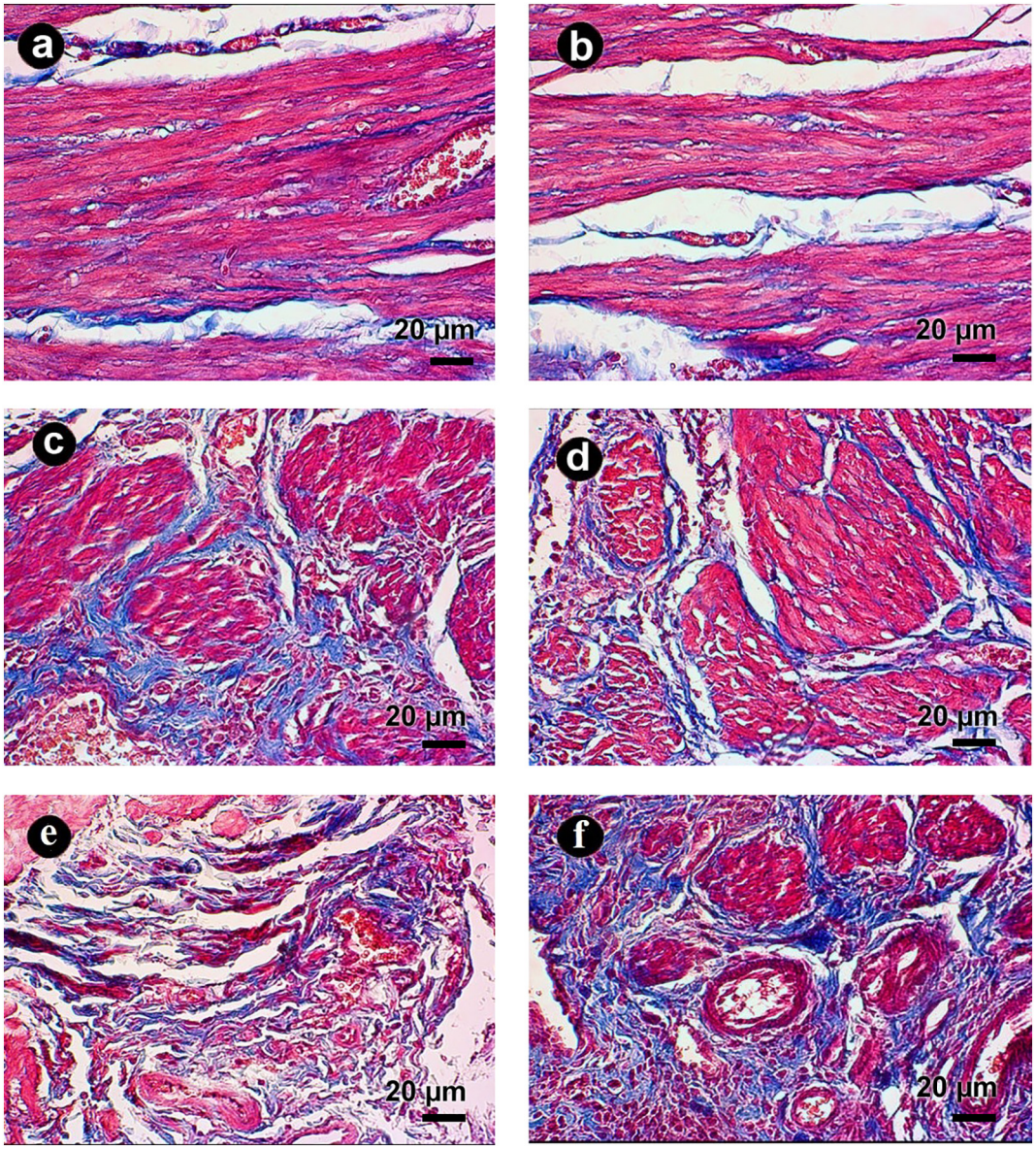

Fig. 6. Masson's trichrome staining. Percentage of the content of bladder smooth muscle (red area) in the control and the experimental group was significantly higher than that in the negative control group. Blue area represents the fibrous connective tissue. (a) Control (b) Sham (c) hSCI, (d) hSCI + BM-MSCs, (e) cSCI (f) cSCI + BM-MSCs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

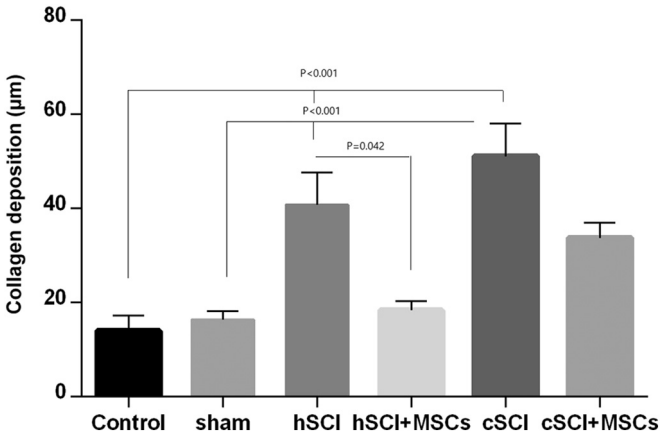

Fig. 7. Changes in collagen area in all groups.

bladder function in cSCI + BM-MSCs group. Besides, two rats in both hSCI and cSCI groups showed infection in the bladder following the SCs transplantation that appropriately responded to the medication with ciprofloxacin for 3 days. This improvement was observed in the first days after treatment. We believed that the results of this study could not completely be applied to human counterpart due to the lack of the cholinergic system in rodent bladder.

To date, there is little data regarding the application of various SCs to restore urinary bladder function post-injuries. The potency of various SC types must be defined related to regenerative potency in the urogenital system. An appropriate cell number, possible interval administration, the golden time for cell transplantation should be addressed. Any genetic manipulations and/or pre-conditioning increasing the regenerative potency of SCs need to be investigated. Considering the current clinical trials available in human medicine, most trials lack well-designed procedure and the advent of comprehensive and sophisticated therapeutic approaches are essential.

5. Conclusion

The MSCs ameliorated bladder dysfunction in the SCI model especially in the hSCI group when transplanted in chronic injury stages. BM-MSCs transplantation into bladder wall may be a novel therapeutic strategy for bladder dysfunction in SCI patients at the chronic phase suffering from NGB disorder. Some issues regarding the transplantation of cell to the human must be addressed prior to extensive application. It seems that MSCs could be touted as a novel magic bullet in the restoration of urinary bladder post-incomplete SCI-injury.

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Conflict of interest

The authors declare no conflicts of interest.

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