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Therapeutic effect of adipose-derived regenerative cells on bladder function in rats with underactive bladder

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ABSTRACT

We examined the effect of adipose-derived regenerative cells (ADRC) on bladder functions in a rat model of detrusor underactivity (DU) induced by bladder over-distention.

Adult female Sprague Dawley rats were divided into 3 groups: sham group (control); over-distention group; and over-distention with ADRC treatment group. Bladder was over-distended with saline (2.7mL) on day 1, 8, 15 and 22 of the study. ADRCs, which were harvested from male F344 rats, expanded via culture, were injected into the bladder wall at day 15. Cystometry and in vitro organ bath functional studies were performed on day 28. Moreover, histological assessment of the bladder was performed.

In cystometry, significant prolongation of the inter-contraction interval (ICI) and decrease of voiding efficiency (VE) were observed in the over-distention group, compared to that in the control group. Significant improvement in ICI and VE was seen in the ADRC treatment group in comparison with the over-distention group. The over-distention group showed significantly weaker bladder contractile responses to carbachol and electrical field stimulation than the control group, while bladder contractile responses were significantly stronger in the ADRC treatment group than that in the over-distention group. The over-distention group showed substantial fibrosis of the bladder compared to the control group, whereas bladder fibrosis was alleviated in the ADRC treatment group.

In conclusion, the injection of ADRC into bladder wall improved bladder dysfunction and histological changes induced by bladder over-distention. ADRCs-based regenerative therapy could be novel treatment for DU.

Keywords: underactive bladder, adipose-derived regenerative cells, over-distention, bladder function, detrusor underactivity

INTRODUCTION

Detrusor underactivity (DU) is defined by the International Continence Society (ICS) as reduced force and/or duration of detrusor muscle contraction, resulting in prolonged micturition and/or failure to achieve complete bladder emptying.[1] In clinical practice, not only bladder

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outlet obstruction (BOO) but also DU were reported to be common causes of lower urinary tract symptoms (LUTS). Epidemiological studies have shown that approximately 30% of LUTS patients have DU.[2] The pathophysiology of DU is complicated and various pathological conditions, including loss of intrinsic contractility, impaired activation of detrusor, early termination of voiding reflex and failure of integration/processing, were thought to induce DU.[2,3] In clinical practice, treatment is required for an underactive bladder induced by detrusor underactivity (UAB/DU). However, there is no effective treatment for UAB/DU at present.

Currently, stem cell therapy is receiving much attention as an effective technique for the regeneration of tissues damaged by several diseases. Mesenchymal stem cell (MSC) therapies, including those with adipose-derived stem cells, muscle-derived stem cells and bone marrow stem cells, are applied in various clinical environments. We believe that autologous adipose-derived regenerative cells (ADRC) therapy may be useful for clinical application of stem cell therapy as safe and low cost nonculture methods, because the human body is rich in adipose tissue, which can be easily and safely harvested in large quantities with minimal morbidity. Periurethral injection of autologous ADRCs in clinical practice was reported to be effective for treating male stress urinary incontinence.[4,5] Additionally, several studies have reported the efficacy of ADRCs in the treatment of ischemic colonic anastomosis, liver injury, myocardial infarction, knee osteoarthritis, and vocal fold paralysis.[6-10]

In the treatment of UAB/DU, autologous muscle-derived cell transplantation was reported to increase muscle contractility in animal models and reduce post-void residual urine volume (PVR) in patients with UAB.[11-14] However, very few studies have evaluated the effect of ADRCs on bladder function in UAB/DU. Based on the background stated above, the objective of the present study was to evaluate the efficacy of ADRCs in changing bladder contractility and tissues, using an animal UAB/DU model.

MATERIAL AND METHODS

All animal experiments were performed in accordance with institutional guidelines and were approved by the Nagoya University Institutional Animal Care and Use Committee.

Animals

Female, 8-10-week-old Sprague Dawley rats weighing 230–260 g (Nihon SLC Co. Ltd., Shizuoka, Japan) were used. UAB/DU was induced by over-distention of the bladder. Specifically, under isoflurane anesthesia, A PE-50 catheter (Clay Adams Division of Becton Dickinson, Parsippany, NJ) was inserted into the bladder via the urethra and connected to an infusion pump. A non-traumatic clamp was applied to the distal urethra and saline was infused at 0.06 mL/min for 45 min. The bladder was over distended by 2.7 ml saline. Clamping was maintained for 15 min following distension. Over-distention was performed 4 times on day 1, 8, 15, and 22 (Fig. 1). In sham operated rats (control), the same operation was conducted, but without saline infusion. The animals were divided into 3 groups: sham operated group (control), over-distention group, and ADRC treatment group after over distension.

Preparation and implantation of ADRC

The isolation and expansion of ADRCs were carried out as previously described.[15] Briefly, inguinal adipose tissue was taken from male F344 rats. After being washed with ice cold phosphate-buffered saline (PBS) at pH 7.4, the adipose tissue was digested with 0.25% collagenase type I (Gibco life technologies, Carlsbad, CA, USA) for 90 min at 37°C with moderate

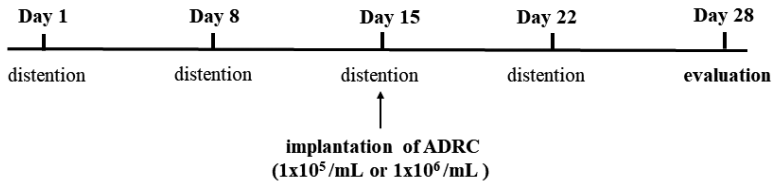

Fig. 1 Study protocol

shaking. Next, the cell suspension was centrifuged at 1000 rpm for 4 min and the pellets were cultured in a low serum medium containing 2% fetal bovine serum (FBS; ICN Biomedical, Aurora, OH, USA) and 10 ng/mL human FGF-2 (Peprotech, Rocky Hill, NJ, USA). To identify adipose derived cells, the harvested cultured cells were labeled, just before implantation, with the PKH26 fluorescent cell linker (Sigma Aldrich, St. Louis, MO), following the manufacturer's protocol. The number of labeled cells and the viability of cells determined by trypan blue exclusion were estimated with a cell counter (Erma, counting chamber counter). Subsequently, labeled cells were resuspended in Dulbecco's modified eagle's medium (DMEM; Gibco life technologies, Carlsbad, CA, USA) containing 2% fetal bovine serum and 10 ng/mL human FGF-2 at 1.0×10^6 cells/mL, 1.0×10^5 cells/mL.

As for intra-detrusor implantation of ADRCs, the rats in ADRC treatment group were anesthetized via inhalation of 4% isoflurane, and a midline abdominal incision was made to expose the bladder. We randomly implanted 100 μl of ADRCs at concentrations of 1.0×10^6 or 1.0×10^5 cells/mL in 4 places in the bladder wall using a 28-gauge syringe, on day 15 (Fig. 1). The following experiments were performed by blinded examiners.

Cystometry

The rats were anesthetized with isoflurane. The bladder was exposed with a lower midline abdominal incision, and PE-50 tubing was inserted into the bladder dome. The catheter was connected to a pressure transducer and an infusion pump using a 3-way stopcock. Cystometry was performed on day 28 under conditions where the rats were conscious and restrained in a recording cage. After 2 hours of accumulation, saline was infused transvesically at 0.04 mL/min and rats voided spontaneously through the urethra. At least 4 reproducible micturition cycles were recorded after an initial stabilization period. Baseline pressure, pressure threshold for voiding, peak voiding pressure, inter-contraction interval (ICI), maximal cystometric capacity, voided volume (VV), residual urine volume (PVR), voiding efficiency (VE), and bladder compliance were evaluated, using the Chart 7 software package (AD Instrument, Milford, MA), as we previously described.[16]

Organ bath study

Following cystometry, the bladder was removed, and immediately immersed in Krebs–Henseleit solution composed of 143 mM Na^+, 5.9 mM K^+, 2.5 mM $Ca2^+$, 1.2 mM $Mg2^+$, 127.7 mM Cl^-, 1.2 mM $SO42^-$, 1.2 mM $PO43^-$, 25 mM $HCO3^-$, and 11 mM glucose, bubbled with 95% O_2 and 5% CO2 to attain a pH of 7.4. Tissue from the bladder body was longitudinally cut into strips approximately 0.5 × 0.5 × 7 mm and weighing 1 to 2 mg. The bladder strip was mounted in a 25 ml organ bath filled with modified Krebs solution and equilibrated in modified Krebs solution for at least 1 h before measurement. Then each preparation was attached to isometric force displacement transducers (Myobath carrier amplifier; World Precision Instruments, Sarasota,

Florida) and the isometric force was recorded and monitored on an ink-writing recorder. Concentration–response curves for carbachol were obtained by increasing the concentration in a step wise manner after response to the previous concentration had reached a plateau.

Electrical field stimulation was performed in preparations mounted between 2 parallel platinum wire electrodes. Electrical impulses for field stimulation of the nervous system of the strips were delivered with a Grass S88 stimulator (Grass Technologies, West Warwick, Rhode Island). Intrinsic nerves were stimulated with rectangular pulses of 0.5 msec duration and 20 V, at stimulation frequencies of 2 to 32 Hz. Trains of pulses lasted 2 s and the stimulation interval was 120 s.

Histological analyses

After post-fixation with 4% paraformaldehyde overnight, bladder tissues were incubated with 20% sucrose for cryoprotection for 48 hours. Thereafter, they were embedded into the OCT compound (Sakura Finetek USA, Inc.) and frozen. Serial sections (8 μm) were cut and stained with hematoxylin and eosin (H&E) and Masson's trichrome using standard methods. To detect nerve structure and muscle structures, sections were stained with antibodies for either S100 or smooth muscle actin (SMA). After blocking with 10% donkey serum, sections were incubated with antibodies for SMA (mouse monoclonal; Dako Denmark) or S100 (1:50 mouse monoclonal; Thermo Fisher scientific) for overnight at 4°C Additionally, the sections rinsed with PBS were incubated with the donkey anti-mouse IgG conjugated with Alexa Fluor 488 (1:250; Molecular Probes, Eugene, OR) for 1 hour at room temperature. All histological sections were observed and photographed using a Keyence BZ-9000 Fluorescence Microscope (Keyence Microsystems, Japan).

Statistical analysis

All statistical values are expressed as mean ± standard deviation. The statistical significance of differences among groups was determined by one-way ANOVA, followed by a post hoc Tukey's test for multiple comparisons. The differences were considered significant at $p < 0.05$. All statistical analyses were performed using SPSS software (IBM, Armonk, NY, USA).

RESULTS

Cystometry

Typical cystometry charts for each group are shown (Fig. 2). In the over-distention group (n = 8), significant prolongation of ICI and decrease of VE were observed, compared with that in the control group (n = 8). In the ADRC treatment group (ADRC 1×10^5/mL group: n = 8, ADRC 1×10^6/mL group: n = 8), significant improvement in ICI and VE was seen, compared with that in the over-distention group, while there was no difference in PVR and VE between the control group and the ADRC treatment group (Table 1).

Functional study

In regard to carbachol and EFS-induced contractions, the contractile strength increased in all groups, as the concentration of carbachol increased and the frequencies of electric stimulus increased. However, the mean concentration and frequency response for smooth muscle strips of the over-distention group (n = 8) showed significantly weaker contraction, compared with that of the control (n = 8). While in the ADRC treatment group, the contractile response was restored adequately and significantly superior in both the high concentration ADRC group (1.0×10^6 cells/mL, n = 8) and the low concentration ADRC group (1.0×10^5 cells/mL, n = 8) than the control group. The contractile response in ADRC group was almost at the same level as that in the control

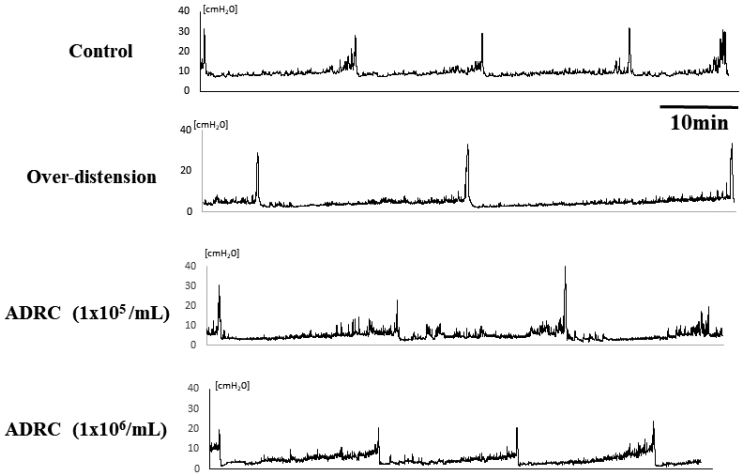

Fig. 2 Cystometry under an awake condition. Representative traces of cystometry in the groups of control, over-distension, and over-distension with ADRC treatment

Table 1 Results of cystometric parameters between control, over-distension, and ADRC groups

	Control (n=8) Mean ± SD	Overdistension (n=8) Mean ± SD	ADRC 1×10^5/mL (n=8) Mean ± SD	ADRC 1×10^6/mL (n=8) Mean ± SD
Inter-contraction interval (sec)	13.4 ± 4.1	24.0 ± 7.3 *	20.1 ± 5.9 *	20.7 ± 6.1 *
Maximum peak pressure (cmH_2O)	36.2 ± 6.5	33.0 ± 9.0	31.0 ± 7.0	33.3 ± 9.4
Post-void residual (mL)	0.02 ± 0.02	0.28 ± 0.27 *	0.06 ± 0.04 * #	0.05 ± 0.03 * #
Voiding efficiency (%)	97 ± 4	64 ± 27 *	85 ± 10 * #	93 ± 5 * # §

* : p <0.05 vs control, # : p <0.05 vs Over-distension, §: p <0.05 vs ADRC 1×10^5/mL

group in both carbachol and EFS-induced contractions study (Fig. 3A, 3B).

Histology of the bladder

H&E and Masson's trichrome stained images for each group are shown (Fig. 4A, 4B). In the over-distention group, fibrosis of smooth muscle in the bladder progressed and the proportion of fibrosis reached approximately 50%, a level that was significantly higher than that in the control group (approximately 5%). Fibrosis of smooth muscle in the bladder was inhibited in the ADRC treatment group and the proportion was lower than that in the over-distention group (Fig. 5). In the immunostaining study of bladder tissue, not only α-SMA positive cells but also S100 positive cells, which represented the presence of nerve structure, were detected in the bladder in ADRC group. Additionally, trichrome staining showed that PKH26 labeled cells merged with α-SMA positive cells and S100 positive cells (Fig. 6).

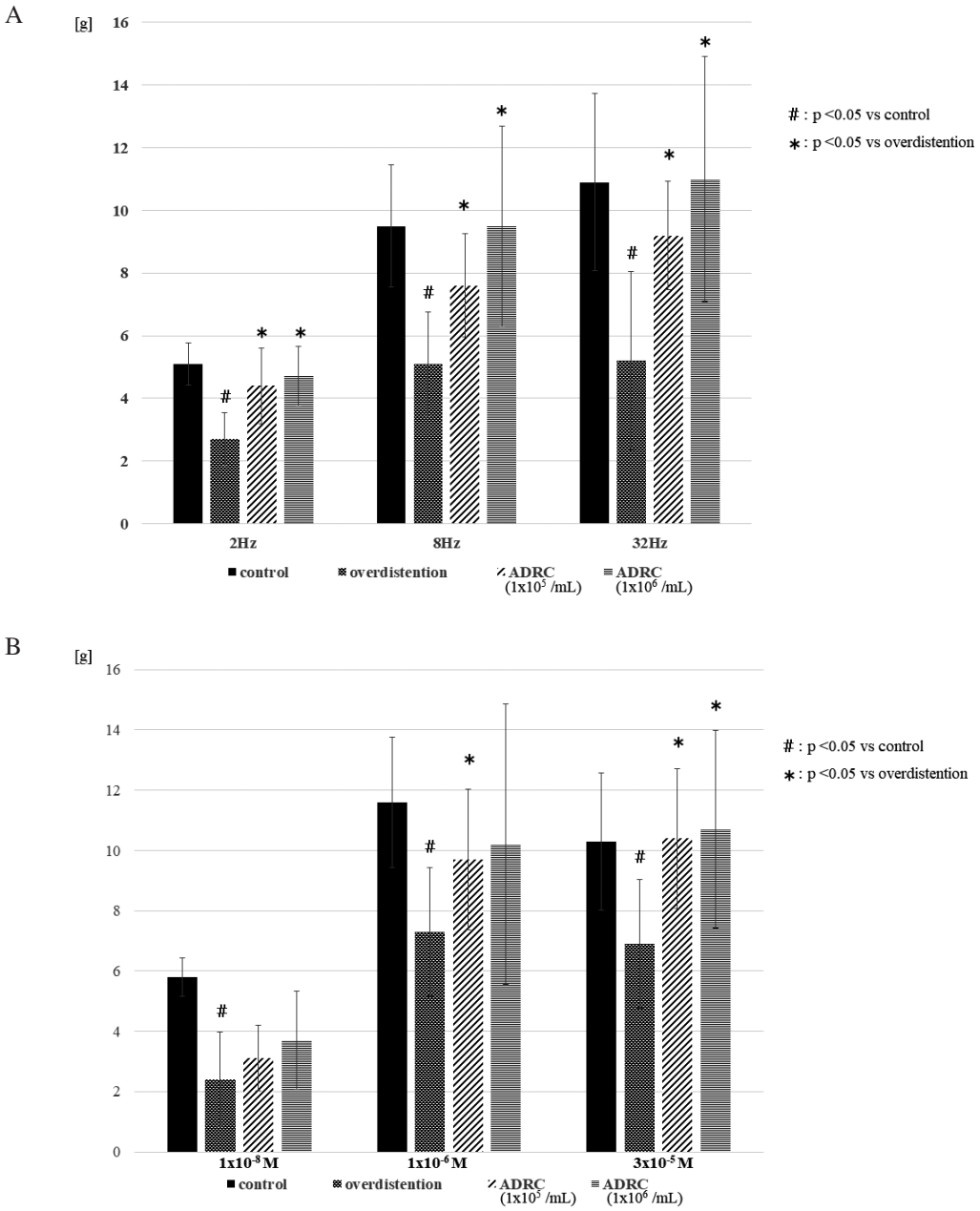

Fig. 3 Functional study using bladder strips

Fig. 3A: Contractile strength of bladder according as the frequencies of electric stimulus in 8 control, 8 over-distension, 8 ADRC (1×10^5 /mL) and 8 ADRC (1×10^6 /mL).

Fig. 3B: Contractile strength of bladder according as the concentration of carbachol in 8 control, 8 over-distension, 8 ADRC (1×10^5 /mL) and 8 ADRC (1×10^6 /mL).

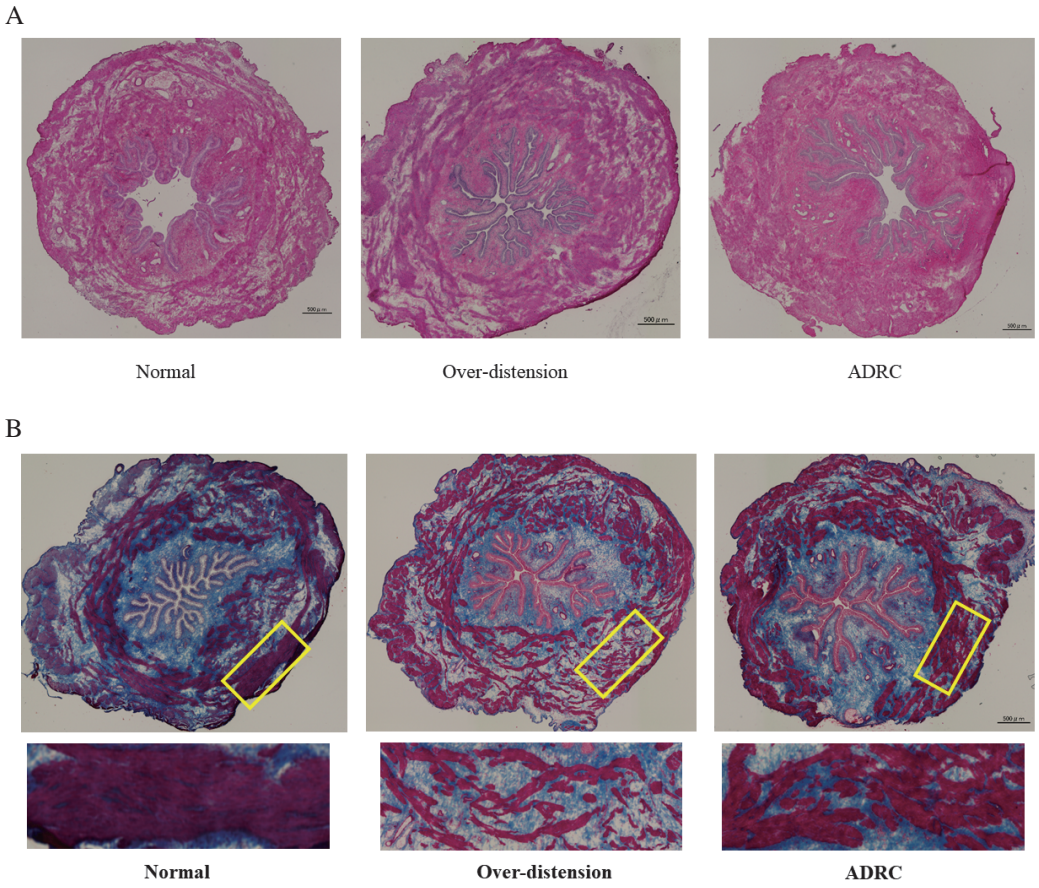

Fig. 4 Histological findings of the bladder between the three groups
Fig. 4A: Hematoxylin and eosin staining of the bladder in control, over-distension, and ADRC
Fig. 4B: Masson's trichrome staining of the bladder in control, over-distension, and ADRC

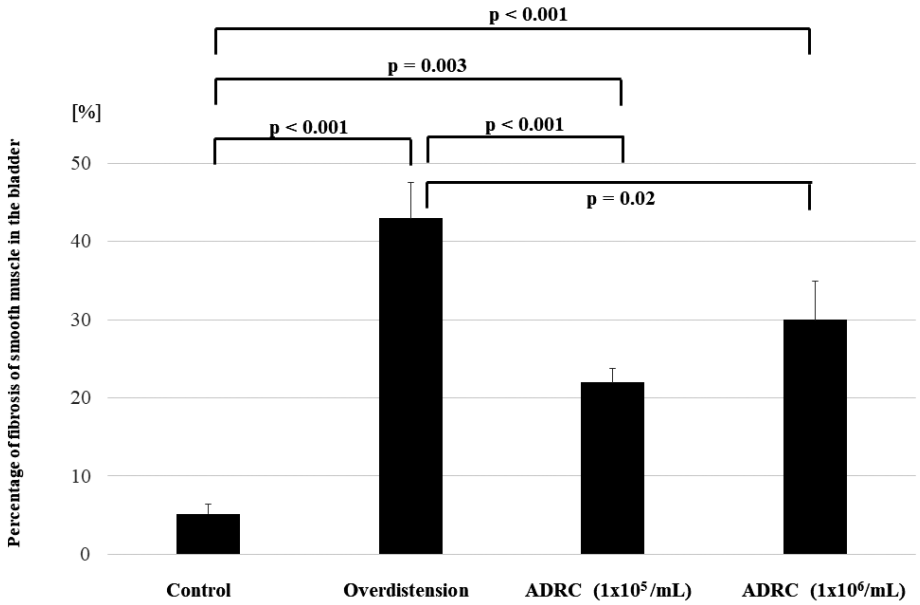

Fig. 5 Percentage of fibrosis in the smooth muscle layer of the bladder in rats: 8 control, 8 over-distension, 8 ADRC (1×10^5 /mL) and 8 ADRC (1×10^6 /mL)

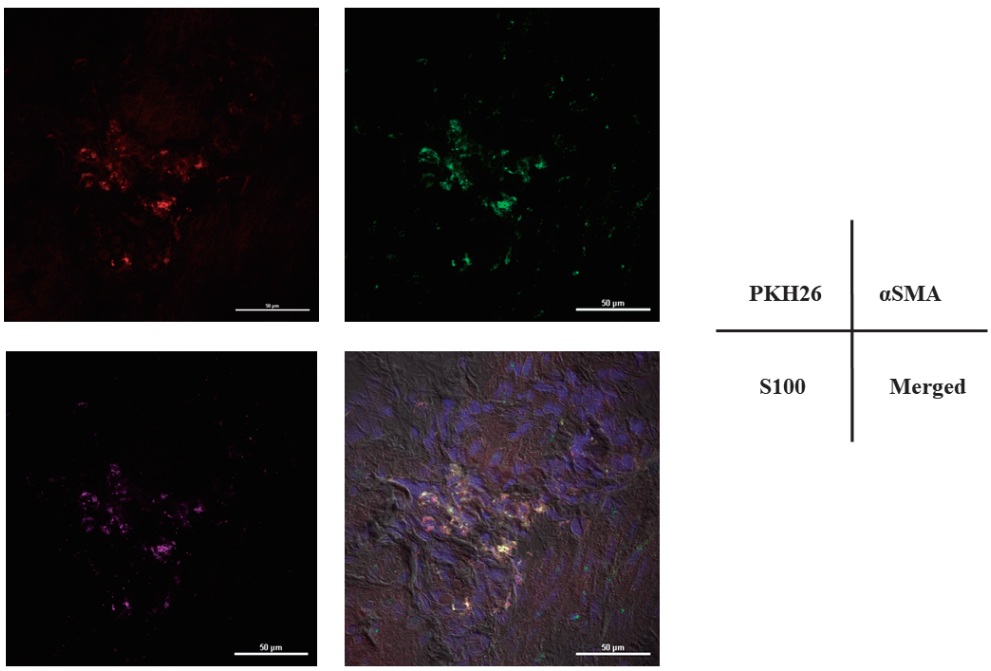

Fig. 6 Bladder tissue stained with antibodies for PKH26, S100, and SMA in ADRC group. Trichrome staining showed that PKH26 labeled cells merged with α-SMA positive cells and S100 positive cells

DISCUSSION

MSC therapies, such as with ADSCs, muscle-derived stem cells, and bone marrow stem cells, are applied in various clinical fields. In the field of urology, MSC therapies were reported to be effective for stress urinary incontinence (SUI) by promoting myogenic differentiation and making a greater contribution to increased urethral resistance.[4,17-19] Recently, we also reported that periurethral injection of ADSCs was a safe and feasible treatment modality with long term efficacy for patients with male stress urinary incontinence caused by urethral sphincter deficiency.[20] In this manner, the effect of MSC therapy on stress urinary incontinence (SUI) was shown in not only basic research but also under clinical conditions. Meanwhile, regarding MSC therapies for UAB/DU, Nishijima et al reported that bone marrow cell transplantation improved bladder contractility in rats with UAB due to bladder outlet obstruction (BOO).[21] Tremp et al reported that injection of ADSCs into bladder and muscle precursor cells prevented pathophysiology remodeling and regenerated bladder tissue and function in rats with UAB/DU induced by BOO.[22] Additionally, Levanovich et al reported a case study in which intradetrusor injection of adult muscle-derived cells decreased bladder capacity measured by cystometry for a 79-year-old man who underwent clean intermittent catheterization for detrusor underactivity.[14] Recently, some studies have reported on the effect of MSC therapies on UAB/DU. However, they are few in number and there were no studies which had methodically evaluated the effects of MSC therapies for UAB/DU, except that of the UAB model induced by BOO.

The animal UAB/DU model was created for the purpose of studying diabetes, aging, bladder outlet obstruction, bladder over-distension, pelvic nerve injury, bladder ischemia, and bladder cryo-injury.[23] We used rat models, whose bladders were over-distended more than once, as UAB/DU models. Rat bladder over-distention models showed significant prolongation of ICI, decrease of VE, and a higher proportion of fibrosis in bladder smooth muscle, compared with those of the control group. In addition, structural changes of the detrusor muscle occurred during over-distension, resulting in an increased connective tissue-to-smooth muscle ratio and decreased SMA expression. The preparation of this model was relatively easy, with less variation in bladder function and histology, and therefore considered to be suitable as an animal DU/UAB model.

First, we evaluated the effect of ADRCs on detrusor contractility in the rat UAB/DU model induced by over-distension of the bladder. A significant improvement of bladder function, including a reduction of PVR and the improvement of voiding efficiency, was seen in the underactive bladder rat, due to injection of ADRCs into the bladder wall. Additionally, histological examination showed that ADRCs underwent differentiation into functional cells such as smooth muscles. In clinical practice, UAB/DU was reported to be present in 20–40% of patients undergoing urodynamic evaluation for non-neurogenic LUTS.[2] However, no effective treatment has been found for this condition. Several studies including ours have indicated that MSC therapies are effective for UAB/DU in various animal models, and therefore show potential as a new, effective treatment for UAB/DU in clinical practice.

One notable aspect of the current study was that injection of ADRCs into the bladder wall contributed to a significant increase in the bladder smooth muscle volume and bladder contractility. The detailed mechanism underlying these functions remains poorly understood, but plausible hypotheses for these finding include the following. First, it was due to direct differentiation of ADRCs to smooth muscle. Successful differentiation of MSCs into smooth muscle has been reported in several studies,[24,25] and we have shown that α-SMA positive cells were detected in the bladder in ADRC group. This suggests that ADRCs underwent differentiation into smooth muscle. Secondly, the injection of ADRCs into the bladder wall have been reported to result in the secretion of many growth factors, including hepatic growth factor, nerve growth factor,

insulin-like growth factor, vascular endothelial growth factor, and tissue growth factor,[26,27] although the levels of various growth factors and cytokines were not evaluated in the current study. Activation of these growth factors may be conducive to the increase of bladder blood flow and tissue regeneration. Consequently, it may be thought of as contributing to the recovery of bladder function.

In the current study, 2 variable doses of ADRCs, at concentrations of 1.0×10^6 or 1.0×10^5 cells/mL, were used in the ADRC group. Although no significant difference in ICI, PVR, and voiding efficiency between the two treatment groups was indicated by cystometry, the contractile response tended to be superior in the high concentration ADRC group (1.0×10^6 cells/mL) than the low concentration ADRC group (1.0×10^5 cells/mL), in the carbachol and EFS-induced contractions study. Thus, ADRC therapy may increase bladder contractility for UAB/DU, in a dose dependent manner, although further studies will be needed in order to determine optimum dosage.

CONCLUSION

Injection of ADRCs into the bladder wall promoted their differentiation into smooth muscle and improved bladder contractility and voiding efficiency in a reliable rat UAB/DU model, induced by bladder over-distention. These findings may be useful to improve bladder function and lower urinary tract symptoms for many UAB/DU patients at the clinical level.

CONFLICT OF INTEREST

This study has been not funded and supported by any company. All authors declare that they have no conflict of interest.

REFERENCES

1. Abrams P, Cardozo L, Fall M, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. *Neurourol Urodyn*. 2002;21:167–178. doi: 10.1002/nau.10052.
2. Osman NI, Chapple CR, Abrams P, et al. Detrusor underactivity and the underactive bladder: a new clinical entity?. A review of current terminology, definitions, epidemiology, aetiology, and diagnosis. *Eur Urol*. 2014;65:389–398. doi: 10.1016/j.eururo.2013.10.015.
3. Yoshida M, Yamaguchi O. Detrusor underactivity: the current concept of the pathophysiology. *Low Urin Tract Symptoms*. 2014;6:131–137. doi: 10.1111/luts.12070.
4. Gotoh M, Yamamoto T, Kato M, et al. Regenerative treatment of male stress urinary incontinence by periurethral injection of autologous adipose-derived regenerative cells: 1-year outcomes in 11 patients. *Int J Urol*. 2014;21:294–300. doi: 10.1111/iju.12266.
5. Vinarov A, Atala A, Yoo J, et al. Cell therapy for stress urinary incontinence: present-day frontiers. *J Tissue Eng Regen Med*. 2018;12:e1108–e1121. doi: 10.1002/term.2444.
6. Yoo JH, Shin JH, An MS, et al. Adipose-tissue-derived stem cells enhance the healing of ischemic colonic anastomoses: an experimental study in rats. *J Korean Soc Coloproctol*. 2012;28:132–139. doi: 10.3393/jksc.2012.28.3.132.
7. Saidi R, Rajeshkumar R, Shariftabrizi A, Zimmerman A, Walter O. Human adipose-derived mesenchymal stem cells promote liver regeneration. *J Invest Surg*. 2015;28:303–308. doi: 10.3109/08941939.2015.1006379.
8. Ishii M, Shibata R, Shimizu Y, et al. Multilayered adipose-derived regenerative cell sheets created by a novel magnetite tissue engineering method for myocardial infarction. *Int J Cardiol*. 2014;175:545–553. doi: 10.1016/j.ijcard.2014.06.034.
9. Lee WS, Kim HJ, Kim KI, Kim GB, Jin W. Intra-articular injection of autologous adipose tissue-derived

mesenchymal stem cells for the treatment of knee osteoarthritis: a phase IIb, randomized, placebo-controlled clinical trial. *Stem Cells Transl Med*. 2019;8:504–511. doi: 10.1002/sctm.18-0122.

10. Nishio N, Fujimoto Y, Suga K, et al. Autologous fat injection therapy including a high concentration of adipose-derived regenerative cells in a vocal fold paralysis model: animal pilot study. *J Laryngol Otol*. 2016;130:914–922. doi: 10.1017/S0022215116008707.
11. Zhang H, Qiu X, Shindel AW, et al. Adipose tissue-derived stem cells ameliorate diabetic bladder dysfunction in a type II diabetic rat model. *Stem Cells and Development*. 2012;21:1391–1400. doi: 10.1089/scd.2011.0244.
12. Jin Y, Xu L, Zhao Y, Wang M, Jin X, Zhang H. Endogenous stem cells were recruited by defocused low-energy shock wave in treating diabetic bladder dysfunction. *Stem Cell Reviews*. 2017;13:287–298. doi: 10.1007/s12015-016-9705-1.
13. Liang CC, Shaw SS, Huang YH, Lin YH, Lee TH. Improvement in bladder dysfunction after bladder transplantation of amniotic fluid stem cells in diabetic rats. *Scientific Reports*. 2018;8:2105. doi: 10.1038/s41598-018-20512-z.
14. Levanovich PE, Diokno A, Hasenau DL, Lajiness M, Pruchnic R, Chancellor MB. Intradetrusor injection of adult muscle-derived cells for the treatment of underactive bladder: pilot study. *Int Urol Nephrol*. 2015;47:465–467. doi: 10.1007/s11255-015-0924-1.
15. Iwashima S, Ozaki T, Maruyama S, et al. Novel culture system of mesenchymal stromal cells from human subcutaneous adipose tissue. *Stem Cells Dev*. 2009;18:533–543. doi: 10.1089/scd.2008.0358.
16. Majima T, Yamamoto T, Funahashi Y, et al. Effect of naftopidil on bladder microcirculation in a rat model of bladder outlet obstruction. *Low Urin Tract Symptoms*. 2017;9:111–116. doi: 10.1111/luts.12119.
17. Carr LK, Robert M, Kultgen PL, et al. Autologous muscle derived cell therapy for stress urinary incontinence: a prospective, dose ranging study. *J Urol*. 2013;189:595–601. doi: 10.1016/j.juro.2012.09.028.
18. Stangel-Wojcikiewicz K, Jarocha D, Piwowar M, et al. Autologous muscle-derived cells for the treatment of female stress urinary incontinence: a 2-year follow-up of a Polish investigation. *Neurourol Urodyn*. 2014;33:324–330. doi: 10.1002/nau.22404.
19. Kuismanen K, Sartoneva R, Haimi S, et al. Autologous adipose stem cells in treatment of female stress urinary incontinence: results of a pilot study. *Stem Cells Transl Med*. 2014;3:936–941. doi: 10.5966/sctm.2013-0197.
20. Gotoh M, Yamamoto T, Shimizu S, et al. Treatment of male stress urinary incontinence using autologous adipose-derived regenerative cells: long-term efficacy and safety. *Int J Urol*. 2019;26:400–405. doi: 10.1111/iju.13886.
21. Nishijima S, Sugaya K, Miyazato M, et al. Restoration of bladder contraction by bone marrow transplantation in rats with underactive bladder. *Biomed Res*. 2007;28:275–280. doi: 10.2220/biomedres.28.275.
22. Tremp M, Salemi S, Largo R, et al. Adipose-derived stem cells (ADSCs) and muscle precursor cells (MPCs) for the treatment of bladder voiding dysfunction. *World J Urol*. 2014;32:1241–1248. doi: 10.1007/s00345-013-1200-6.
23. Tyagi P, Smith PP, Kuchel GA, et al. Pathophysiology and animal modeling of underactive bladder. *Int Urol Nephrol*. 2014;46:S11–21. doi: 10.1007/s11255-014-0808-9.
24. Watanabe T, Maruyama S, Yamamoto T, et al. Increased urethral resistance by periurethral injection of low serum cultured adipose-derived mesenchymal stromal cells in rats. *Int J Urol*. 2011;18:659–666. doi: 10.1111/j.1442-2042.2011.02795.x.
25. Nagaya N, Kangawa K, Itoh T, et al. Transplantation of mesenchymal stem cells improves cardiac function in a rat model of dilated cardiomyopathy. *Circulation*. 2005;112:1128–1135. doi: 10.1161/CIRCULATIONAHA.104.500447.
26. Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation*. 2004;109:1292–1298. doi: 10.1161/01.CIR.0000121425.42966.F1.
27. Hirose Y, Funahashi Y, Matsukawa Y, et al. Comparison of trophic factors secreted from human adipose-derived stromal vascular fraction with those from adipose-derived stromal/stem cells in the same individuals. *Cytotherapy*. 2018;20:589–591. doi: 10.1016/j.jcyt.2018.02.001.