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Synergistic Effect of Mesenchymal Stem Cells Infected with Recombinant Adenovirus Expressing Human BDNF on Erectile Function in a Rat Model of Cavernous Nerve Injury

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Purpose: To evaluate the combined role of mescenchymal stem cells (MSCs) infected with recombinant adenoviruses expressing human BDNF (rAd/hBDNF) on the erectile dysfunction in rat with cavernous nerve injury.

Materials and Methods: Rats divided into 4 groups: control group, bilateral cavernous nerve crushing group (BCNC group), BCNC with MSCs group and BCNC with MSCs infected with rAd/hBDNF group. After 4-week, functional assessment was done. PKH26 and BDNF staining of major pelvic ganglion and masson's trichrome staining of corpus cavernosum were performed. Western blot analysis of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) was done in corpus cavernosum.

Results: After 4 weeks, BCNC with MSCs and MSCs infected with rAd/hBDNF groups showed significantly well-preserved erectile function compared with BCNC group. Moreover, the erectile function of MSCs infected with rAd/hBDNF group was significantly well-preserved than BCNC with MSCs group. The smooth muscle of corpus cavernosum was significantly preserved in BCNC with MSCs and MSCs infected with rAd/hBDNF groups compared with BCNC group. More preservation of smooth muscle was observed in rats with MSCs infected with rAd/hBDNF than with MSCs alone. Significant increase expression of eNOS and nNOS was noted in rats with MSCs infected with rAd/hBDNF than with MSCs alone.

Conclusions: The erectile function was more preserved after injection with MSCs infected with rAd/hBDNF in rat with ED caused by cavernous nerve injury. Therefore, the use of MSC infected with rAd/hBDNF may have a better treatment effect on ED cause by cavernous nerve injury.

Key Words: *Brain-derived growth factor; Erectile dysfunction; Stem cells*

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INTRODUCTION

At present, the number of radical prostatectomy procedures has been increasing because of the increased detection of prostate-confined disease owing to earlier diagnosis by prostate-specific antigen screening. The results of a study about the risk factors of prostate cancer in North America showed that 94% of patients with prostate cancer have clinically localized disease [1]. Erectile dysfunction (ED) is a common complication following radical prostatectomy, and about 20 to 30% of patients may not return to normal erectile function at 12 months after surgery [2-4]. ED is a significant complication that has a negative impact on the patient's quality of life. It is especially important to pre-

vent this complication because the number of radical prostatectomy procedures in relatively younger patients with normal preoperative potency has been increasing [5]. Therefore, nerve-sparing radical prostatectomy with preservation of the cavernous nerve was introduced to help in recovery of erectile function following surgery. Bilateral nerve sparing shows superior spontaneous recovery of erectile function compared with that after surgery without nerve sparing [6-8]. However, 20 to 80% of these patients may never return to normal erectile function despite undergoing bilateral nerve-sparing radical prostatectomy [9]. Therefore, several studies have been conducted to explore methods for restoring the normal function of the penile neurovasculature after cavernosal nerve injury.

As our understanding of the molecular and biological factors and mechanisms of ED has grown, management of ED with growth factors or stem cells has appeared as a new treatment method that offers the potential to reverse the underlying causes of ED [10]. Among the various neurotrophins, brain-derived neurotrophic factor (BDNF) has shown a specific, important role in penile nerve recovery in a rat model of cavernous nerve injury [11]. Also, there are many reports that stem cell-based therapies seem to be able to repair damaged penile tissue by neuronal, vascular, or muscular implantation and differentiation [10]. However, the effects of combination therapies with BDNF and stem cells have not yet been studied. Therefore, the aim of this study was to examine the effect of mescenchymal stem cells (MSCs) infected with recombinant adenovirus expressing human BDNF (rAd/hBDNF) on erectile function in a rat model of cavernous nerve injury.

MATERIALS AND METHODS

1. Animals

White male Sprague-Dawley rats (weighing 300 to 350 g) were obtained from Samtako Inc. (Osan, Korea). The rats were divided into 4 groups: control (n=10), bilateral cavernous nerve crushing (BCNC group, n=10), BCNC followed by injection with MSCs (BCNC with MSCs group, n=10), and BCNC followed by injection with MSCs infected with rAd/hBDNF (MSCs infected with rAd/hBDNF group, n=10). During the experiments, the animals had free access to water and normal food. The experimental protocol was approved by the Catholic University Animal Ethics Committee (CUMC-2009-0045-01), and the animals were handled according to the National Institutes of Health guidelines.

2. Preparation of MSCs expressing hBDNF

rAd/hBDNF was constructed as previously described [12] by using the AdEasy Vector System (QBiogene, Carlsbad, CA, USA). For preparation of rat bone marrow-derived MSCs (rBM-MSCs), bone marrow cells were collected from the femurs and tibias of 3- to 4-week-old SD rats by flushing respective tissues with Hank's balanced salt solution (WelGENE, Daegu, Korea) containing 2% fetal bovine serum (Hyclone, Logan. UT, USA). After red blood cells were removed, bone marrow cells were filtered through a 40-μm cell strainer (BD Bioscience, San Jose, CA, USA) and separated by using Ficoll density gradient centrifugation. Isolated bone marrow cells were resuspended and cultured in Dulbecco's modified Eagle medium (1,000 mg/l glucose; WelGENE) with 20% fetal bovine serum, 100 U/ml penicillin, and 100 g/ml of streptomycin (Invitrogen, Carlsbad, CA, USA) for about 10 days until colonies formed. Colonies were harvested and used for subsequent experiments as MSCs. For animal experiments, *ex vivo* expanded MSCs (less than passage 5) were seeded into a 70-mm flask at an initial density of 1.4×10^6 cells and incubated overnight at 37°C. The cells were infected with a mixture of 0.1 μM 4HP412 and rAd/hBDNF or rAd/hIGF-1 (50 multiplicity of infection) and incubated at 37°C for 30 minutes [12]. Cells were then trypsinized, washed with phosphate-buffered saline (PBS), and administered to rats (1×10^6 cells/rat).

3. BCNC and MSC-rAd/hBDNF administration

Tiletamine (Zoletil) 0.2 ml was injected intraperitoneally to anesthetize the animals. A lower midline incision was made and the prostate gland was exposed. After identification of the major pelvis ganglion (MPG) on the lateral side to bilateral prostates, the cavernous nerves, tracking posterolaterally, were identified and isolated. In the control group (sham surgery), no further surgical manipulation was done. In the remaining groups, the cavernosal nerves were isolated and a crush injury was induced by using a hemostat clamp for 2 minutes. In the BCNC group, the abdomen was closed after the bilateral cavernosal nerve crushing. In the two treatment groups, MSCs (1×10^6 in 20 μl) and MSCs (1×10^6 in 20 μl) infected with rAd/hBDNF were administered into the MPG in each group after BCNC.

4. Erectile functional assessment

The rats were anesthetized with an intraperitoneal injection of 0.2 ml tiletamine (Zoletil). With the rat in the supine position, the penis was dissected and the corpus cavernosum and crus of the penis were exposed. A low, midline abdominal incision was made to access the pelvis, and the MPG lateral to the right prostate was exposed. For the measurement of intracavernosal pressure (ICP), a heparinized 23G butterfly needle was inserted in the corpus cavernosum of the penile proximal portion after the penile skin was degloved and the corpus cavernosum identified. Then a bipolar electrical stimulator was placed on the ganglion to stimulate the cavernosal nerve for 50 seconds at 1.5 mA, 20 Hz, pulse width 0.2 ms. The cavernosal nerve stimulation was conducted at least 3 times and the interval between stimulations was maintained for over 10 minutes. At the completion of functional analysis, the MPG and penis were excised for histopathology.

5. Immunofluorescent staining of BDNF

Immediately following measurement of ICP, the ganglion

was removed. The tissue was then snap frozen by using 2-methylbutane pre-cooled in liquid nitrogen. Cryostat sections of the ganglion were embedded in tissue-Tek OCT (Sakura Finetechnical Co., Ltd., Tokyo, Japan). From each ganglion, consecutive sections (5 μm) were collected on 4 to 5 slides, and thus each contained a similar collection of 10 to 15 serial sections from the same animal.

Ganglion sections were washed three times for 5 minutes with PBS, and to avoid nonspecific antibody binding, they were then incubated for 60 minutes with 2% normal goat serum (Chemicon International, Temecula, CA, USA) containing 0.1% Triton X-100. The samples were incubated with anti-BDNF (diluted to 1:200; Abcam, Cambridge, UK) overnight at 4°C in a humidified chamber. Sections were then incubated with the secondary antibody (Alexa Fluor 488 goat anti-rabbit IgG; Invitrogen) for 2 hours in the dark in a humidified chamber at room temperature. After washing (3 times, 10 minutes each) with PBS, the nuclei were counterstained with 4,6-diamino-2-phenylindole dihydrochloride (DAPI; Vector Labs, Burlingame, CA, USA). Immunofluorescence was visualized by using an Olympus BX51 fluorescence microscope (Olympus Co., Tokyo, Japan).

6. Masson's trichrome staining

After cavernosometry, the skin-denuded middle part of the penile shafts were fixed overnight in 10% formalin, washed, and stored in 70% alcohol at 4°C until processed for paraffin-embedded tissue sectioning (5 μm). The cavernosal tissue was obtained for the Masson's trichrome staining. After staining, the color distribution of the muscle tissue was approximated by using Adobe Photoshop CS 8.0. After the entire color distribution of the image was calculated, we selected the muscle tissue distribution, expressed as the color red. There were somewhat standard deviations in our calculation because of color overlays and ambiguity of the color spectrum of the muscle tissues.

7. eNOS and nNOS protein expression by Western blot

The skin, dorsal vein, and urethra of all rats were removed. The corpus cavernosum was obtained from all rats and homogenized individually in a buffer solution of 0.32 M sucrose, 0.2 M Hepes, (pH 7.4), 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, 10 μg/ml leupeptin, 2 μg/ml aprotinin, 1 μg/ml pepstatin, 10 μg/ml trypsin inhibitor, and 1 mM phenylmethyl sulfonyl fluoride. The homogenized buffer solution was placed on ice for 15 minutes and centrifuged at 4°C and 13,000 rpm for 15 minutes. The supernatant solution was separated. The separated solution was utilized in the bovine serum albumin. Amounts of 30 μg of the quantitative protein was denatured at 95°C for 5 minutes and electrophoresis was performed on a 12% discontinuous sodium dodecylsulfate-polyacrylamide gel. The proteins were then electroblotted onto a 0.2 μm polyvinylidenedifluoride (Amersham Bioscience, Piscataway, NJ, USA) membrane for 150 minutes at 25 V. The membranes were reacted with blocking buffer (5% skim milk in TBS-T buffer) for 30 minutes at the ambient temperature.

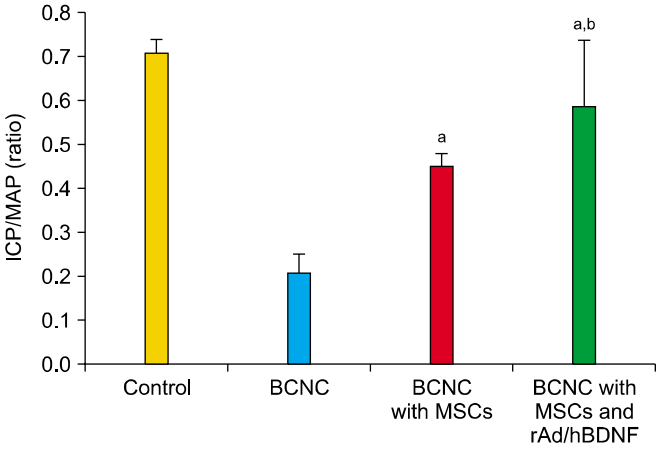

FIG. 1. Assessment of erectile function by ICP tracing under cavernous nerve stimulation at 4 weeks after BCNC in rats administered MSCs only or MSCs infected with rAd/hBDNF. ICP, intracavernosal pressure; MAP, mean arterial pressure; BCNC, bilateral cavernous nerve crushing; MSCs, mesenchymal stem cells; rAd/hBDNF, recombinant adenovirus expressing human brain-derived neurotrophic factor. [a]:p=0.05 compared with the BCNC group, [b]:p=0.05 compared with the BCNC injected with MSCs group.

The eNOS and nNOS (BD Biosciences) antibodies were added for 2 hours, and the membrane was washed 3 times by using TTBS at intervals of 10 minutes. As the secondary antibodies, anti-mouse IgG-HRP and anti-goat IgG-HRP (1:2000 dilution; Zymed Laboratories, San Francisco, CA, USA) were added at the ambient temperature for 1 hour and the membrane was washed again with TTBS for 6 times with an interval of 5 minutes between each washing. Chemiluminescence was detected by using enhanced chemiluminescence (ECL) Western blotting detection reagents. Densitometric assessment of the bands on the autoradiogram was performed using Bio1D ver. 97 (Vilber Lourmat, Marne La Vallée, France).

8. Statistical analysis

All measurements were expressed as means±standard deviations. Statistical analysis was performed by use of Sigma Stat 3.0 for Windows (Systat Software Inc., San Jose, USA). An inter-group comparison was made with the use of Neumann-Keuls multiple comparison test. The cut-off value of statistical significance was $p < 0.05$.

RESULTS

1. Erectile function assessment

Erectile function was measured at 4 weeks after the operation. The analysis is presented as the ratio of ICP to mean arterial pressure (ICP/MAP; Fig. 1). The ICP/MAP ratios in the control group were 0.71±0.03, which was significantly higher than in all other groups ($p < 0.05$). The ICP/MAP ratio was dramatically decreased to 0.21±0.04 in the BCNC group ($p < 0.05$). The ICP/MAP ratios of the

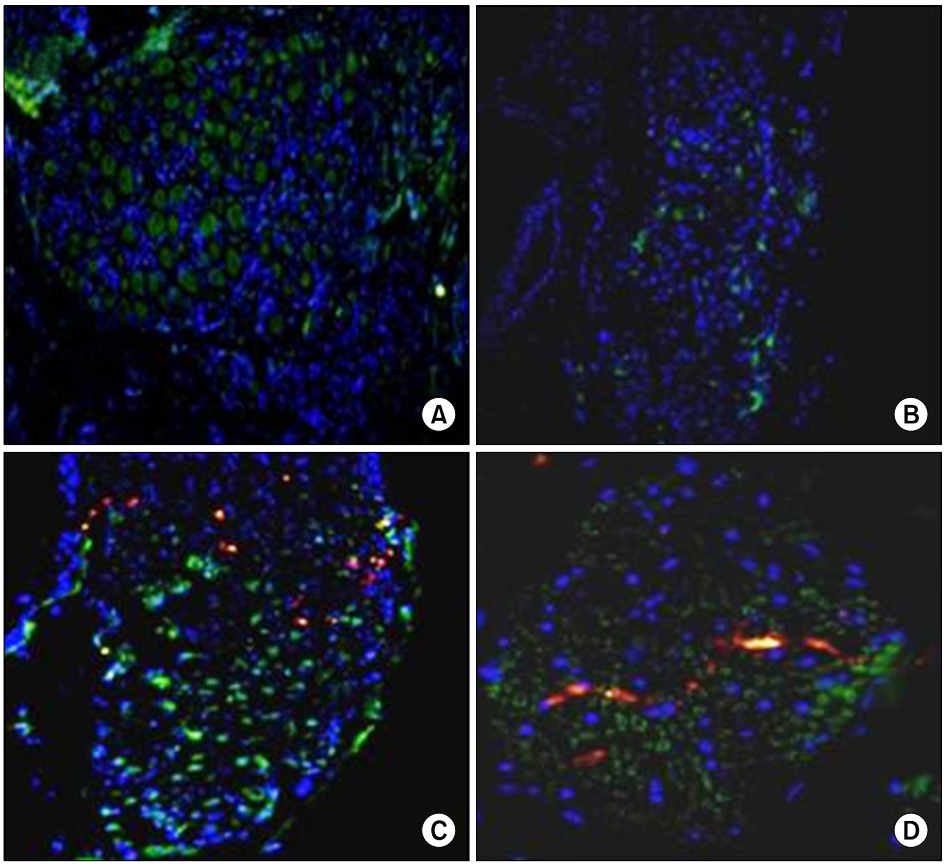

FIG. 2. Expression of brain-derived neurotrophic factor (BDNF) in major pelvic ganglion at 4 weeks in the control group (A), BCNC group (B), BCNC with MSCs group (C), and BCNC with MSCs infected with rAd/hBDNF group (D) (×40; blue, cell nucleus; green, BDNF; red, MSCs). BCNC, bilateral cavernous nerve crushing; MSCs, mesenchymal stem cells; rAd/hBDNF, recombinant adenovirus expressing human brain-derived neurotrophic factor.

BCNC with MSCs group (0.45±0.03) and the BCNC with MSCs infected with rAd/BDNF group (0.59±0.15) were significantly higher than that of the BCNC group (0.25±0.02, $p < 0.05$). Moreover, the ICP/MAP ratio of the BCNC with MSCs infected with rAd/BDNF group (0.65±0.03) was significantly higher than that of the BCNC with MSCs group (0.25±0.02, $p < 0.05$).

2. Expression of BDNF in MPG by immunofluorescent staining

Immunofluorescent staining of BDNF of MPGs in the BCNC group was remarkably decreased compared with that in the control group (Fig. 2). BDNF in the BCNC with MSCs and BCNC with MSCs infected with rAd/hBDNF groups showed a significant increase in expression compared with the BCNC group. A slightly greater increase in expression of BDNF was observed in the BCNC with MSCs infected with rAd/hBDNF group than in the BCNC with MSCs group.

3. Smooth muscle/collagen ratio of corpus cavernosum

The staining with Masson's trichrome in the BCNC group revealed a significantly decreased smooth muscle/collagen ratio (0.15±0.12) compared with that in the control group (0.57±0.09, $p < 0.05$; Fig. 3). The smooth muscle/collagen ratios of the BCNC with MSCs (0.36±0.13) and BCNC with MSCs infected with rAd/hBDNF (0.47±0.17) groups were significantly increased compared with the BCNC group ($p < 0.05$). In addition, a significantly increased smooth muscle/collagen ratio was observed in the BCNC with MSCs infected with rAd/hBDNF group compared with the BCNC group ($p < 0.05$).

4. Quantification of eNOS and nNOS proteins

Decreased expression of eNOS and nNOS was observed in the BCNC group than the control group ($p < 0.05$, Fig. 4). The expression of eNOS and nNOS was significantly increased in the BCNC with MSCs infected with rAd/hBDNF group compared with the BCNC group ($p < 0.05$). In addition, significantly increased expression of eNOS and nNOS was observed in the BCNC with MSCs infected with rAd/hBDNF group than in the BCNC group ($p < 0.05$).

DISCUSSION

In this study, we report the synergistic effects of MSCs and rAd/hBDNF on the restoration of erectile function in ED induced by cavernous nerve injury. The preservation of erectile function was identified by both functional and morphological studies.

Stem cell therapy using MSCs derived from bone marrow is an attractive treatment for tissue regeneration and engineering owing to the differentiation potentials and hypoimmunogenic properties of MSCs [13]. In addition, MSCs have tropism to inflammatory sites such as damaged tissue and tumors [14,15]. Therefore, several studies using MSCs

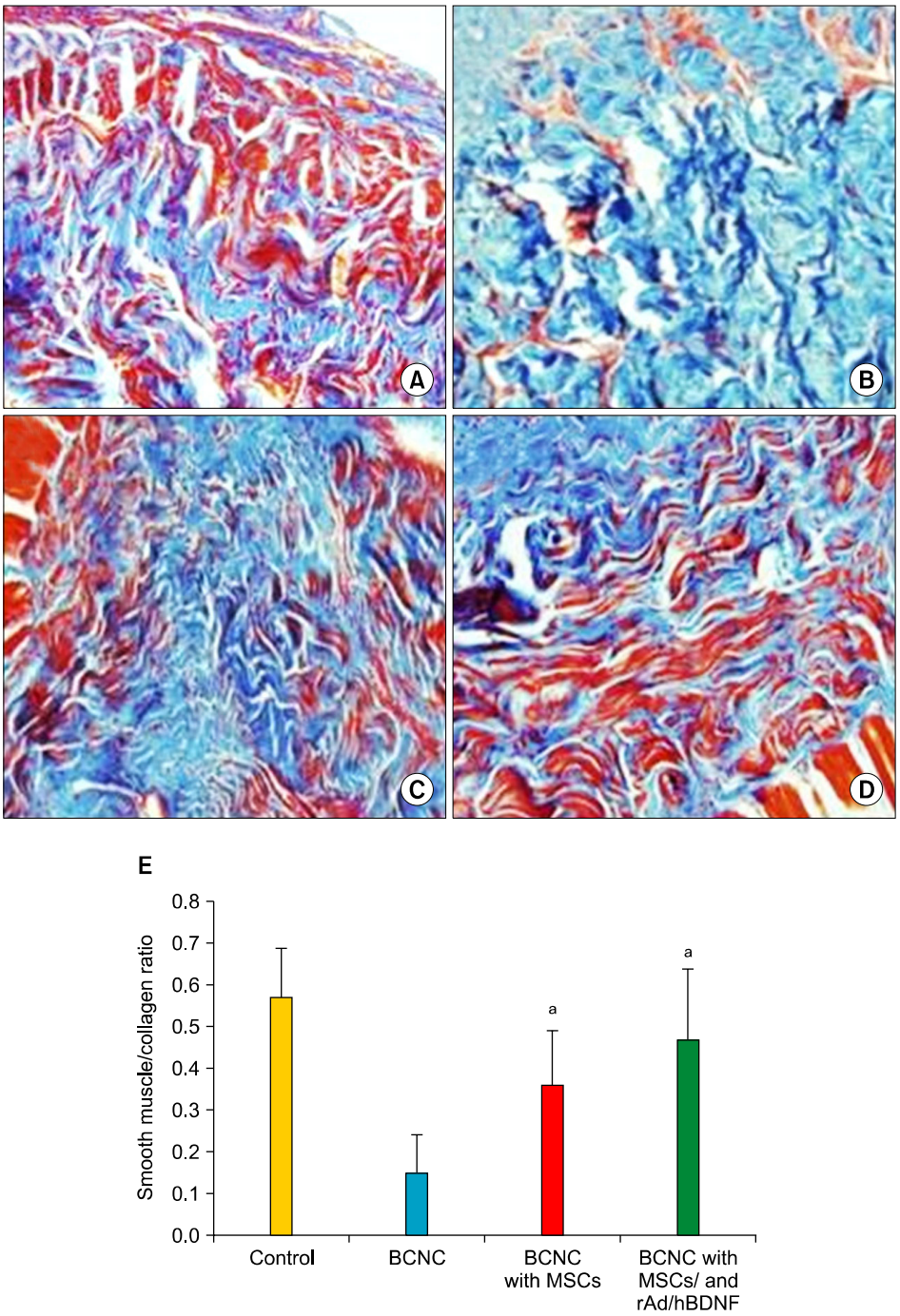

FIG. 3. Smooth muscle/collagen ratios at 4 weeks in the control group (A), BCNC group (B), BCNC with MSCs group (C), and BCNC with MSCs infected with rAd/hBDNF group (D) and smooth muscle/collagen ratios in the control, BCNC, BCNC with MSCs, and BCNC with MSCs mixed with Matrixen (E). BCNC, bilateral cavernous nerve crushing; MSCs, mesenchymal stem cells; rAd/hBDNF, recombinant adenovirus expressing human brain-derived neurotrophic factor. [a]:$p < 0.05$ compared with the BCNC group.

have reported a restoration of erectile function in animals with ED of various causes, for example, diabetes and cavernous nerve injury. In this study, MSCs alone were expected to have a role in the restoration of erectile function. The ICP/MAP ratios in rats administered MSCs were significantly greater than the ratios in the untreated cavernous nerve injury rats. Furthermore, a relative increase in expression of BDNF in the MPG and better preservation of cavernous smooth muscle were observed in the rats administered MSCs. These results were similar to those of other studies that evaluated the effect of various stem cells, such as muscle-derived stem cells or adipose-derived stem cells, on neurogenic ED [10]. Considering these results, we propose that erectile function after cavernous nerve injury can be preserved owing to the regenerative effect of MSCs by differentiation into neural tissue in the MPG [16].

The important role of endogenous neurotrophins on neuroprotection has been studied in neurogenic ED induced by cavernous nerve injury. Neurotrophins such as BDNF, growth differentiation factor-5, and neutrin have been shown to have neuromodulatory abilities on neuronal survival after cavernous nerve injury in previous studies [17]. For example, the central neuromodulatory role of BDNF has been reported after cavernous nerve injury. According

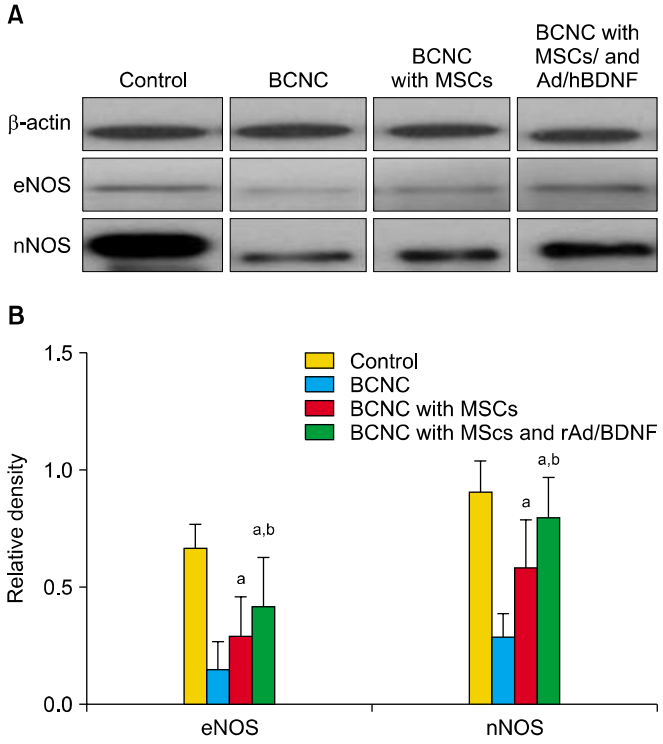

FIG. 4. (A) Western blot analysis of endothelial nitric oxide synthase (eNOS) and neuronal NOS (nNOS) expression in the corpus cavernosum at 4 weeks in the control group, BCNC group, BCNC with MSCs group, and BCNC with MSCs infected with rAd/hBDNF group. (B) Densimetric analysis to β-actin of eNOS and nNOS. BCNC, bilateral cavernous nerve crushing; MSCs, mesenchymal stem cells. [a]:$p < 0.05$ compared with BCNC group, [b]:$p < 0.05$ compared with the BCNC with MSCs group.

to a study using MPG and proximal cavernosal nerves isolated from male rats, a significant outgrowing of neurites is observed after administration of exogenous BDNF; moreover, it was proved that this phenomenon occurs via the Janus Kinase (JAK)/signal transducer and activator of transcription (STAT) molecular pathway [18,19]. The JAK/STAT pathway transduces cellular signals directly from the cell membrane to the nucleus and is known to modulate central and peripheral nerve regeneration. Moreover, up-regulation of endogenous BDNF is observed in the MPG and cavernous smooth muscle after cavernous nerve transaction [20]. In response to cavernous nerve transaction, the mRNA and protein expression of BDNF is significantly elevated in the MPG in a time-dependent manner by activation of the JAK/STAT pathway. The BDNF level is elevated at 24 hours and maintained for 5 days after injury. However, we observed that the expression of BDNF was significantly decreased in the MPG at 4 weeks after injury. This change in BDNF may be due to factors related to time. The level of BDNF in the MPG seems to increase to recover the injury in the early period after cavernous nerve injury. Humans have the ability to self-reproduce; however, the injured tissue might not be recovered by the self-reproduction mechanism according to the degree of injury or period of the injured state. In this study, we assessed the degree of expression of BDNF during a longer period after cavernous nerve injury than in the previous study. This difference in the observation period may have resulted in the decrease in the BDNF level in this study. Therefore, a chronic state of neurogenic ED may have a negative neurobiological effect on the self-recovery response and may be a cause of ED after cavernous nerve injury. However, further study of the sequential changes of the BDNF level after cavernous nerve injury is needed to support this suggestion.

In this study, the ICP/MAP ratios in rats administered MSCs with BDNF were significantly higher than the ratios in rats administered MSCs alone and in BCNC rats. In addition, the increased expression of BDNF in the MPG was observed in the both the BCNC with MSCs and BCNC with MSCs infected with rAd/hBDNF groups. Furthermore, administration of MSCs infected with rAd/hBDNF resulted in a greater increase in expression of BDNF than in the group administered MSCs only. Together with the change in BDNF in the MPG, elevated levels of eNOS and nNOS were observed in the corpus cavernosum of the two treated groups at 4 weeks after injury. In particular, nNOS was significantly elevated after administration of MSCs infected with rAd/hBDNF. This finding is similar to the study by Bakircioglu et al. [11], who demonstrated that BDNF enhanced recovery of erectile function and regeneration of nNOS-containing nerve fibers after injury. As a result, we suggest that the combination treatment of MSCs and BDNF has a synergistic effect on the recovery of ED after cavernous nerve injury. In addition, several studies have shown the important role of BDNF in the neuronal differentiation of MSCs. Bone marrow MSCs also express BDNF and nerve growth factor (NGF) by themselves, and these neurotrophins help bone marrow MSCs to differentiate into neuronal cells [21,22]. Yaghoobi and Mahani [23] observed that both BDNF and NGF expression decrease following differentiation and that the subtraction of these two important neurotrophins may become a drawback for neurally differentiated cells in recovery of the damaged nervous tissue. As a result, BDNF may be a significant factor in the neuronal differentiation of MSCs as well as in the restoration of damaged nervous tissue. These results suggest that combination treatments with MSCs and BDNF may have a more effective role on the recovery of neurogenic ED than treatment with MSCs alone.

Despite the promising roles of combination treatment with MSCs and BDNF shown in this study, there is a limitation to clinical application. The safety of the adenovirus used as a vector for BDNF introduction to MSCs has not been established in humans; therefore, further studies are necessary.

CONCLUSIONS

In this study, combination treatment with MSCs and BDNF resulted in better functional and histological preservation in ED after cavernous nerve injury than did treat-

ment with MSCs alone. This synergistic effect may have resulted from the additive role of BDNF on the differentiation of MSCs into neuronal tissue. With further evaluation of the safety of vectors such as adenovirus in humans, stem cell therapy combined with BDNF may be a possible option for the treatment of ED caused by radical prostatectomy.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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REFERENCES

1. Shao YH, Demissie K, Shih W, Mehta AR, Stein MN, Roberts CB, et al. Contemporary risk profile of prostate cancer in the United States. J Natl Cancer Inst 2009;101:1280-3.
2. Anastasiadis AG, Salomon L, Katz R, Hoznek A, Chopin D, Abbou CC. Radical retropubic versus laparoscopic prostatectomy: a prospective comparison of functional outcome. Urology 2003;62: 292-7.
3. Roumeguere T, Bollens R, Vanden Bossche M, Rochet D, Bialek D, Hoffman P, et al. Radical prostatectomy: a prospective comparison of oncological and functional results between open and laparoscopic approaches. World J Urol 2003;20:360-6.
4. Krambeck AE, DiMarco DS, Rangel LJ, Bergstralh EJ, Myers RP, Blute ML, et al. Radical prostatectomy for prostatic adenocarcinoma: a matched comparison of open retropubic and robot-assisted techniques. BJU Int 2009;103:448-53.
5. Stephenson RA, Mori M, Hsieh YC, Beer TM, Stanford JL, Gilliland FD, et al. Treatment of erectile dysfunction following therapy for clinically localized prostate cancer: patient reported use and outcomes from the Surveillance, Epidemiology, and End Results Prostate Cancer Outcomes Study. J Urol 2005;174:646-50.
6. Walsh PC, Partin AW, Epstein JI. Cancer control and quality of life following anatomical radical retropubic prostatectomy: results at 10 years. J Urol 1994;152(5 Pt 2):1831-6.
7. Haffner MC, Landis PK, Saigal CS, Carter HB, Freedland SJ. Health-related quality-of-life outcomes after anatomic retropubic radical prostatectomy in the phosphodiesterase type 5 ERA: impact of neurovascular bundle preservation. Urology 2005;66:371-6.
8. Raina R, Pahlajani G, Agarwal A, Zippe CD. Early penile rehabilitation following radical prostatectomy: Cleveland clinic experience. Int J Impot Res 2008;20:121-6.
9. Zippe CD, Pahlajani G. Penile rehabilitation following radical prostatectomy: role of early intervention and chronic therapy. Urol Clin North Am 2007;34:601-18, viii.
10. Harraz A, Shindel AW, Lue TF. Emerging gene and stem cell therapies for the treatment of erectile dysfunction. Nat Rev Urol 2010;7:143-52.
11. Bakircioglu ME, Lin CS, Fan P, Sievert KD, Kan YW, Lue TF. The effect of adeno-associated virus mediated brain derived neurotrophic factor in an animal model of neurogenic impotence. J Urol 2001;165(6 Pt 1):2103-9.
12. Park SH, Doh J, Park SI, Lim JY, Kim SM, Youn JI, et al. Branched oligomerization of cell-permeable peptides markedly enhances the transduction efficiency of adenovirus into mesenchymal stem cells. Gene Ther 2010;17:1052-61.
13. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 2000;28:875-84.
14. Sasaki M, Abe R, Fujita Y, Ando S, Inokuma D, Shimizu H. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. J Immunol 2008;180:2581-7.
15. Aboody KS, Brown A, Rainov NG, Bower KA, Liu S, Yang W, et al. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci U S A 2000;97:12846-51.
16. Levy YS, Bahat-Stroomza M, Barzilay R, Burshtein A, Bulvik S, Barhum Y, et al. Regenerative effect of neural-induced human mesenchymal stromal cells in rat models of Parkinson's disease. Cytotherapy 2008;10:340-52.
17. Bella AJ, Lin G, Lin CS, Hickling DR, Morash C, Lue TF. Nerve growth factor modulation of the cavernous nerve response to injury. J Sex Med 2009;6 Suppl 3:347-52.
18. Bella AJ, Lin G, Tantiwongse K, Garcia M, Lin CS, Brant W, et al. Brain-derived neurotrophic factor (BDNF) acts primarily via the JAK/STAT pathway to promote neurite growth in the major pelvic ganglion of the rat: part I. J Sex Med 2006;3:815-20.
19. Lin G, Bella AJ, Lue TF, Lin CS. Brain-derived neurotrophic factor (BDNF) acts primarily via the JAK/STAT pathway to promote neurite growth in the major pelvic ganglion of the rat: part 2. J Sex Med 2006;3:821-7.
20. Bella AJ, Lin G, Garcia MM, Tantiwongse K, Brant WO, Lin CS, et al. Upregulation of penile brain-derived neurotrophic factor (BDNF) and activation of the JAK/STAT signalling pathway in the major pelvic ganglion of the rat after cavernous nerve transection. Eur Urol 2007;52:574-80.
21. Yaghoobi MM, Mowla SJ. Differential gene expression pattern of neurotrophins and their receptors during neuronal differentiation of rat bone marrow stromal cells. Neurosci Lett 2006;397:149-54.
22. Qu R, Li Y, Gao Q, Shen L, Zhang J, Liu Z, et al. Neurotrophic and growth factor gene expression profiling of mouse bone marrow stromal cells induced by ischemic brain extracts. Neuropathology 2007;27:355-63.
23. Yaghoobi MM, Mahani MT. NGF and BDNF expression drop off in neurally differentiated bone marrow stromal stem cells. Brain Res 2008;1203:26-31.