SEXUAL MEDICINE

Intravenous Infusion of Bone Marrow–Derived Mesenchymal Stem Cells Reduces Erectile Dysfunction Following Cavernous Nerve Injury in Rats

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ABSTRACT

Introduction: Intravenous preload (delivered before cavernous nerve [CN] injury) of bone marrow–derived mesenchymal stem cells (MSCs) can prevent or decrease postoperative erectile dysfunction (J Sex Med 2015;12:1713–1721). In the present study, the potential therapeutic effects of intravenously administered MSCs on postoperative erectile dysfunction were evaluated in a rat model of CN injury.

Methods: Male Sprague-Dawley rats were randomized into 2 groups after electric CN injury. Intravenous infusion of bone marrow–derived MSCs (1.0×10^6 cells in Dulbecco's modified Eagle's medium 1 mL) or vehicle (Dulbecco's modified Eagle's medium 1 mL) was performed 3 hours after electrocautery-induced CN injury.

Main Outcome Measures: To assess erectile function, we measured intracavernous pressure at 4 weeks after MSC or vehicle infusion. Histologic examinations were performed to investigate neuronal innervation and inhibition of smooth muscle atrophy. Green fluorescent protein–positive bone marrow–derived MSCs were used for cell tracking. To investigate mRNA expression levels of neurotrophins in the major pelvic ganglia (MPGs), quantitative real-time polymerase chain reaction was performed.

Results: The decrease of intracavernous pressure corrected for arterial pressure and area under the curve of intracavernous pressure in the bone marrow–derived MSC group was significantly lower than that in the vehicle group at 4 weeks after infusion ($P < .05$). Retrograde neuronal tracing indicated that the MSC group had a larger number of FluoroGold-positive neurons in the MPGs compared with the vehicle group. The ratio of smooth muscle to collagen in the MSC group was significantly higher than in the vehicle group. Green fluorescent protein–positive bone marrow–derived MSCs were detected in the MPGs and injured CNs using confocal microscopy, indicating homing of cells to the MPGs and injured CNs. Brain-derived neurotrophic factor and glial cell-derived neurotrophic factor expression levels in the MPGs were significantly higher in the MSC group than in the vehicle group ($P < .01$).

Conclusion: Intravenous infusion of bone marrow–derived MSCs after CN injury might have therapeutic efficacy in experimental erectile dysfunction. **Matsuda Y, Sasaki M, Kataoka-Sasaki Y, et al. Intravenous Infusion of Bone Marrow–Derived Mesenchymal Stem Cells Reduces Erectile Dysfunction Following Cavernous Nerve Injury in Rats. Sex Med 2017;X:XXX–XXX.**

Key Words: Mesenchymal Stem Cell; Cavernous Nerve Injury; Erectile Dysfunction

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INTRODUCTION

Postoperative erectile dysfunction (ED) after radical prostatectomy (RP) can occur due to surgical injury of the cavernous nerves (CNs), which can adversely affect quality of life.[1,2] Although numerous techniques to preserve CNs during RP have been developed using transperineal, retropubic, or laparoscopic approaches and a minimally invasive robotic procedure,[3] postoperative ED still occurs in a certain population of patients.[4]

Takayanagi et al[5] reported that intravenous infusion of bone marrow–derived mesenchymal stem cells (MSCs) before crushed CN injury can prevent or decrease postoperative ED in a rat

model of CN injury.[5] They demonstrated the proof of principle that intravenous infusion of bone marrow–derived MSCs could have therapeutic efficacy for ED from CN injury. The potential mechanisms might include the distribution of preloaded (delivered before CN injury) bone marrow–derived MSCs to the lesion area, where they could provide neuroprotection by secreting neurotrophins and prevent ED after RP.[5]

In the present study, we tested the hypothesis that intravenous administration of bone marrow–derived MSCs after CN injury would decrease postoperative ED. The MSCs were intravenously infused at 3 hours after CN injury induction. We used an electrocautery-induced CN injury model that might be more clinically relevant compared with mechanical injury models. Evaluation of physiologic changes monitoring intracavernous pressure (ICP) corrected for arterial pressure (AP) was performed to assess the potential inhibition of postoperative ED. Histologic examination including retrograde tracing with FluoroGold (FG) and smooth muscle content also was performed. Quantification of neurotrophic factors using quantitative real-time polymerase chain reaction (RT-PCR) was carried out to assess the potential therapeutic mechanism used by the infused MSCs.

AIM

The aim of this study was to clarify the effect of a cellular therapy to inhibit postoperative ED by systemic administration of MSCs after CN injury in rats.

METHODS

All experiments were carried out in accordance with the institutional guidelines of Sapporo Medical University (Sapporo, Japan). The use of animals in this study was approved by the animal care and use committee of Sapporo Medical University.

Preparation of MSCs From Bone Marrow

MSCs were cultured as described in our previous studies.[6–9] Briefly, bone marrow was collected from the femoral bones of adult wild-type and green fluorescence protein (GFP)-expressing rats (W-Tg [CAG-GFP] 184Ys), diluted to 25 mL with Dulbecco's modified Eagle's medium (DMEM; Sigma, St Louis, MO, USA), supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific Inc, Waltham, MA, USA), L-glutamine 2 mmol/L (Sigma), penicillin 100 U/mL, and streptomycin 0.1 mg/mL (Thermo Fisher Scientific), and incubated for 3 days (5% CO_2 at 37°C). When the cultures almost reached confluence, the adherent cells were detached using a solution of trypsin and ethylenediaminetetra-acetic acid (Sigma) and sub-cultured on a 150-mm^2 tissue culture dish (surface area = 148 cm^2; 1030-150; IWAKI, Tokyo, Japan) at 5×10^5 cells/mL in culture medium 14 mL; thus, the plating density was approximately $3.4 \times 10^3/cm^2$. The surface antigen phenotype of the MSCs was $CD45^-$, $CD73^+$, $CD90^+$, and $CD106^-$.[10] The cultured MSCs were used for transplantation after 3 passages. For infusion, the supernatants were washed out and MSCs (1.0×10^6 cells) were suspended in fresh DMEM 1 mL and injected.

Electrocautery-Induced CN Injury

Male Sprague-Dawley rats weighing 250 to 350 g were anesthetized by intraperitoneal injection with pentobarbital (45 mg/kg). After the abdomen was shaved, a lower midline incision was made in the supine position. Then, the prostate gland, major pelvic ganglia (MPGs), and CNs were exposed. Bilateral CNs (approximately 5 mm distal to the MPGs) were injured by electrical coagulation for 0.5 second with a bipolar forceps connected to a generator (Kirwan 26-1500 Bipolar Coagulator; Kirwan Surgical Products, Marshfield, MA, USA). The bipolar coagulation was performed carefully at 50 kHz and 16 W to prevent cutting the CNs and complete elimination of erectile function. The abdominal incision was closed with a suture and the rats were allowed to recover from anesthesia.

Experimental Protocol

The experimental protocol used is shown in Figure 1. The 1st step was the induction of electrocautery CN injury. 3 hours after electrocautery-induced CN injury, the rats were randomized into MSC and vehicle groups. Rats were intravenously infused with MSCs (1.0×10^6 cells in fresh DMEM 1 mL) or with vehicle (fresh DMEM 1 mL only) through the right external jugular vein. Detection of GFP-expressing bone marrow–derived MSCs (GFP-MSCs) was performed at day 1 and quantitative RT-PCR was performed at day 2. Histologic evaluations were performed after 4 weeks of MSC or vehicle infusion.

Evaluation of Erectile Function

Erectile function was evaluated 4 weeks after MSC or vehicle infusion as described previously[5] (n = 6 per group). Briefly, under pentobarbital (45 mg/kg) anesthesia, bilateral CNs were re-exposed through a lower abdominal midline incision. A scrotal incision was made and the left penile crus was exposed. To measure ICP, a 23-gauge butterfly needle attached to a PE50 (#427400; Becton Dickinson, Franklin Lakes, NJ, USA) tube with heparinized saline (250 IU/mL) was placed at the left penile crus. AP was monitored through a PE50 tube inserted in the right carotid artery. The exposed CN (approximately 3 mm distal to the MPG) was stimulated with a stainless steel electrode (TF-206-011; Unique Medical Co, Tokyo, Japan) connected to an isolated constant-current electrical stimulation device (20 Hz and 1.5 mA; SEN-3301; Nihon Kohden, Tokyo, Japan) for 60 seconds and the changes in ICP and AP were recorded by a pressure transducer. After 3 minutes, the same procedure was performed for stimulation of the right CN. The averaged values were used for further analysis. In the present study, erectile function was assessed with maximal ICP corrected for AP (ICP/AP) and the area under the curve of ICP plotted for 1 minute of stimulation (ICP-AUC) using LabChart (AD Instruments Inc, Colorado Springs, CO, USA).