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**札幌医科大学**  
 Sapporo Medical University

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**Intravenous preload of mesenchymal stem cells rescues  
erectile function in a rat model of cavernous nerve injury**

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Manuscripts

Review

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3 **Intravenous preload of mesenchymal stem cells rescues erectile function in a rat**  
4 **model of cavernous nerve injury**  
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3 **Running head:** *Preload of MSC infusion rescues erectile function in cavernous nerve*  
4  
5 *injury*  
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9 **Take Home Message:**

10 Intravenous delivery of mesenchymal stem cells (MSCs) prior to a cavernous nerve  
11 (CN) injury model for experimental erectile dysfunction (ED), resulted in increased  
12 intracavernous pressure (ICP) / arterial pressure (AP), neuronal innervation and  
13 higher levels of GDNF and Neurturin mRNA in the major pelvic ganglia (MPG).  
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**Abstract***Introduction:*

We evaluated the potential preventive effects and mechanisms of intravenously preloaded mesenchymal stem cells (MSCs) for erectile dysfunction (ED) in a cavernous nerve (CN) injury model.

*Methods:*

Male Sprague Dawley rats were used for this study. Rats were randomized into two groups. One group was intravenously preloaded with MSCs ( $1.0 \times 10^6$  cells in 1 ml total fluid volume) and the other was infused with medium alone (1ml DMEM) for sham control, respectively. Crushed CN injury was induced immediately after infusion. The surgeon was blind to the experimental conditions (MSC or medium).

*Main Outcome Measures:*

To assess erectile function, we measured the intracavernous pressure (ICP) and arterial pressure (AP) at 1 hour and 2 weeks after CN injury. After measuring the initial ICP/AP of pre-injury (normal) male SD rats, they were randomized into the two groups and infused with MSCs or medium. PKH26-labelled MSCs were used for tracking. To investigate the mRNA expression levels of neurotrophins in the major pelvic ganglia (MPG), we performed real-time quantitative RT-PCR.

*Results:*

The reduction of ICP/AP and area under the curve of ICP (ICP-AUC) in the MSC group was significantly lower than in the DMEM group ( $P < 0.05$ ;  $P < 0.05$ ) at one hour. The ICP/AP and ICP-AUC at 2 weeks post-injury in the MSC group was significantly higher than in the DMEM group ( $P < 0.01$ ;  $p < 0.05$ ). The preloaded PKH26-labelled MSCs were detected in the MPG and CN using confocal microscopy indicating homing of the cells to the injured nerve and ganglia. GDNF and neurturin, which are

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3 important neurotrophic factors for erection, had expression levels in MPG  
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5 significantly higher in the MSC group than in the DMEM group ( $P < 0.01, 0.05$ ).  
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8 *Conclusion:* Intravenous preload of MSCs before a CN injury may prevent or reduce  
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10 experimental ED.  
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For Peer Review

## 1. Introduction

Radical prostatectomy (RP) is a recommended curative procedure for patients with low and intermediate risk in localized prostate cancer and is associated with a life expectancy of more than 10 years<sup>1</sup>. Erectile dysfunction (ED) following RP, however, is the major problem and it can reduce patients' quality of life (QOL) after surgery<sup>2,3</sup>. The etiology of ED after RP is considered to be multifactorial<sup>4-6</sup>, but a neurogenic etiology resulting from cavernous nerve (CN) injuries during a nerve-sparing procedure is important<sup>7</sup>.

Despite the progress of nerve sparing techniques that include spreading the robot-assisted procedures, recent post-operative ED rates at one year ranged from 10 to 46%<sup>8</sup>. Common drug therapy is oral intake of phosphodiesterase type 5 inhibitors, but the success rate is significantly lower in this population<sup>9</sup>. Penile rehabilitation is also beneficial treatment for these populations, but the effectiveness is limited and it takes several months to years to recover<sup>4</sup>. Therefore, it is an important research issue to develop approaches to better reduce post-operative ED in patients.

Mesenchymal stem cells (MSCs) derived from adult bone marrow are considered to secrete a number of neurotrophic factors, cytokines and anti-inflammatory molecules that provide neuroprotection, induction of axonal regeneration/sprouting, remyelination and immunomodulation<sup>10</sup>. Much work in animal models and clinical trials have shown that intravenous infusion of MSCs following peripheral nerve injury including sciatic<sup>11</sup> and recurrent laryngeal<sup>12</sup> nerve injuries and central nervous diseases such as stroke<sup>13-15</sup> and spinal cord injury<sup>16</sup> is safe and improves functional recovery. In this study we evaluated the effects of systemically delivered MSCs in an experimental ED model in the rat.

## 2. Aim

The aim of this study is to find a cell therapy approach to prevent or reduce post-operative ED using intravenous delivery of MSCs prior to induction of CN injury in the rat.

## 3. Materials and Methods

### 3.1. *Preparation of mesenchymal stem cells from rat bone marrow*

The use of animals in this study was approved by the animal care and use committee at Sapporo Medical University (#12-030), and all procedures were carried out in accordance with institutional guidelines. Methodology of MSC culture was based upon our previous studies<sup>17</sup>. Briefly, bone marrow was obtained from femoral bones in adult Sprague–Dawley rats (male, 200-250 g), 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 (FBS) (Thermo Fisher Scientific Inc., Waltham, MA, USA), 2 mM l-glutamine (SIGMA), 100 U/ml penicillin, 0.1 mg/ml streptomycin (Thermo Fisher Scientific Inc.) and incubated for 3 days (5% CO<sub>2</sub>, 37 °C). When cultures almost reached confluence, the adherent cells were detached with trypsin-EDTA solution (SIGMA) and subcultured at 1 × 10<sup>4</sup> cells/ml. In the present study, we have used MSCs after three passages.

### 3.2. *Cavernous nerve injury model and assessment of erectile function*

Male Sprague–Dawley rats weighing 250–350 g were anesthetized with pentobarbital (45 mg/kg). Through a lower abdominal midline incision, bilateral major pelvic ganglia (MPG) and CNs were identified and exposed. The CNs



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3 (approximately 5 mm distal to the MPG) were first crushed using a micro needle  
4 holder (FD231R, B. Braun Aesculap Japan, Tokyo, Japan) for 15 sec as previously  
5 described **with minor modification**<sup>18</sup>. The procedure was carried out carefully so as  
6 not to cut the CNs to avoid major injury causing deterioration of erectile function.  
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8 Abdominal wounds were then closed with sutures, and animals were allowed to  
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10 recover from anesthesia.  
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16 The intracavernous pressure (ICP) and arterial pressure (AP) were measured using  
17 the methods similar to those previously reported<sup>19-22</sup>. Animals were anesthetized  
18 with pentobarbital (45 mg/kg). The skin of the left neck was incised and a PE50  
19 (#427400, Becton Dickinson, MD, USA) tube was inserted into the left carotid artery  
20 to monitor AP. Bilateral MPG and CNs were exposed as described above and the  
21 incision was extended to the skin around the penis. A 23G needle connected to a PE50  
22 tube filled with heparinized saline (250 IU/ml) was inserted into the left penile crus.  
23 Electrostimulation (20 Hz and 1.5mA for 60 seconds) of the CNs was applied with a  
24 bipolar hook electrode (TF-206-011, Unique Medical Co., Tokyo, Japan), and  
25 changes of ICP were measured. Maximal ICP, mean AP and area under the curve of  
26 ICP (ICP-AUC) during the period of electrostimulation were analyzed using LabChart  
27 (ADInstruments Inc., Colorado Springs, CO, USA). The maximal ICP value divided  
28 by simultaneous mean AP (ICP/AP) and ICP-AUC were used to evaluate erectile  
29 function.  
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### 49 3.3. Experimental protocol (Fig. 1)

50 First, we measured the initial ICP/AP from pre-injury rats. The rats were  
51 randomized into experimental and sham control groups (MSC and DMEM). Rats  
52 were intravenously infused with MSCs ( $1.0 \times 10^6$  cells in 1ml total fluid volume) and  
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3 medium alone (fresh 1 ml DMEM only) respectively via right external jugular vein.  
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5 CN injury was immediately induced after preload of MSCs or DMEM. The order of  
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7 delivering MSC or DMEM was randomized. The surgeon (A.T.) did not know  
8  
9 whether MSC or DMEM was administered before and during infusion. We measured  
10  
11 ICP/AP at 1 hour and 2 weeks after CN injury. Each ICP/AP measurement at 1 hour  
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13 (n=12/group) and 2 weeks (n=10/group) was a terminal experiment in a separate  
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16 group.  
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#### 20 21 3.4. Retrograde tracing study

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23 Fluorogold (FG: Hydroxystilbamidine, methanesulfonate, H-22845, Thermo  
24  
25 Fisher Scientific Inc.) injection was performed one week before histological  
26  
27 examination as previously reported<sup>19, 22, 23</sup>. Briefly, animals were anesthetized with  
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29 pentobarbital (45 mg/kg). The skin beside the penis was narrowly opened and 4µl of  
30  
31 FG (4.0%) was injected into the right penile crus by using a 30G Hamilton syringe.  
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33 After the ICP measurement the right MPG was collected and immediately fixed in 4%  
34  
35 paraformaldehyde (PFA) for about 48 hours, and then immersed in 0.1 mol/L  
36  
37 phosphate buffer (PB) containing 25% sucrose.  
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41 Quantification of retrograde labeled FG positive cells was performed as  
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43 previously described.<sup>20, 24, 25</sup> Serial frozen transverse sections (14 µm thick) of the  
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45 entire MPG were cut with a cryostat. Every sixth section was mounted on 3  
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47 aminopropyltriethoxysilane-coated slides (12–14 sections per animal). Images of each  
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49 MPG were taken under fluorescent microscope through a wideband UV filter using  
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51 40x objective. We evaluated all FG positive neuronal profiles that had nuclei in each  
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53 MPG section to avoid double-counting of neurons (10 rats each in the MSC and the  
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55 DMEM groups). The standardized selection of sections through the entire MPG and  
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3 counting all FG positive cells on each section allowed an unbiased assessment of  
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5 retrogradely labelled cells between the MSC and DMEM groups spanning the entire  
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7 MPG.  
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### 10 11 3.5. *Detection of PKH26 labeled MSCs*

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14 Cells were prelabeled with the membrane dye PKH26 as previously reported<sup>10</sup>.  
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16 Intracardiac perfusion (4 % PFA) (n=10) were performed immediately after CN injury  
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18 to collect MPGs and CNs and store at -80 °C until use. Transverse cryostat sections  
19  
20 (20 µm) were obtained through the lesion area. Immunohistochemistry was performed  
21  
22 using anti-neurofilament (#N4142, Sigma; 1:500). The sections were examined using  
23  
24 a confocal microscopy with Ex/Em (405; 488; 561: LSM780 ELYRA system; Carl  
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26 Zeiss, Oberkochen, Germany).  
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### 32 3.6. *Real-Time Polymerase Chain Reaction*

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34 One hour after nerve injury, animals (n=5/group) were anesthetized, and the  
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36 MPGs were collected in each group using a microscope. Total RNA was extracted  
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38 using a RNeasy Plus Mini kit (#74134, Qiagen, Valencia, CA, USA) according to the  
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40 manufacturer's instructions<sup>21,25</sup>. The concentration of the RNA was quantified by  
41  
42 determination of optical density at 260 nm. The RNA (1µg) was reverse transcribed  
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44 into cDNA using SuperScript III reverse transcriptase (Qiagen) and nucleotide oligo-  
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46 dT. Real-time polymerase chain reaction (PCR) for each sample was performed in  
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48 triplicates with TaqMan Universal Master Mix II with UNG (Thermo Fisher  
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50 Scientific Inc.). Specific sets of primers and TaqMan probes were purchased from  
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52 Thermo Fisher Scientific Inc.: glyceraldehyde-3-phosphate dehydrogenase (GAPDH)  
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54 (TaqMan rodent GAPDH control reagents, Rn01775763-g1) as an endogenous control,  
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3 GDNF (Rn00569510\_m1) and Neurturin (Rn01527513-g1) as target genes. The  
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5 reactions were run in an ABI-StepOne real-time PCR system (Thermo Fisher  
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7 Scientific Inc.) using the 48-well plate format. The cycling conditions included an  
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9 initial phase at 95°C for 3 min, followed by 40 cycles at 95°C for 15 s, and 55°C for  
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11 60 s. Relative quantification of target gene expression was calculated using the  
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13 comparative threshold cycle method according to the guidelines of the manufacturer.  
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### 18 19 3.7. Statistical analysis

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21 All statistical analyses were performed using SPSS 18 (SPSS, Inc., IL, USA). For  
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23 multiple comparisons, we used Kruskal-Wallis one-way analysis of variance followed  
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25 by Bonferroni *post hoc* test. Comparison between two groups was performed using  
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27 the Mann-Whitney *U*-test.  
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## 32 4. Results

### 33 34 4.1. Erectile function after MSC transplantation

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36 To assess the erectile function, a physiological study with ICP/AP and ICP-AUC  
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38 analyses was carried out. Representative waveforms of the ICP and AP at pre-injury  
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40 in the MSC group (Fig. 2A), pre-injury in the DMEM group (Fig. 2B), 1 hour after  
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42 injury in the MSC group (Fig. 2C) and 1 hour after injury in the DMEM group (Fig.  
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44 2D) are shown in Fig. 2. The ICP/AP and ICP-AUC were significantly decreased one  
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46 hour after injury in both groups (Fig. 2E, 2F:  $P < 0.01$ ). The reduction of ICP/AP in the  
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48 MSC group (54.7%) was 12.4% lower than in the DMEM group (67.1%) at 1 hour  
49  
50 after transplantation. (Fig. 2E,  $P < 0.05$ ). The reduction of ICP-AUC in the MSC group  
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52 (60.2%) was 12.3% lower than in the DMEM group (72.5%) at 1 hour after  
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54 transplantation (Fig. 2F,  $P < 0.05$ ).  
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3 Representative waveforms of the ICP and AP at 2 weeks after transplantation in  
4 the MSC group (Fig. 3A) and the DMEM group (Fig. 3B) are shown in Fig. 3. It  
5 should be noted that waveform of the MSC group (Fig. 3A) shows similar trends as  
6 the one of pre-injury state (Fig. 2A). No change of the waveform in the DMEM  
7 groups at both 1 hour (Fig. 2D) and 2 weeks (Fig. 3B) was observed. The ICP/AP in  
8 the MSC group ( $0.65\pm 0.04$ ) was 41.3% higher than in the DMEM group ( $0.46\pm 0.04$ )  
9 at 2 weeks after transplantation (Fig. 3C,  $P<0.01$ ). The ICP-AUC in the MSC group  
10 ( $5695\pm 553$  cmH<sub>2</sub>O) was 53.7% higher than in the DMEM group ( $3705\pm 527$ cmH<sub>2</sub>O)  
11 at 2 weeks after transplantation. (Fig. 3D,  $P<0.05$ ). The MSC-preloaded group  
12 displayed less ED shortly after CN injury (Fig.2) and erectile function evaluated with  
13 physiological analyses reached near normal values over the time course of two weeks  
14 (Fig.3). Collectively, these results indicate that the intravenous preload of MSCs  
15 prevented or reduced ED after RP.

#### 31 32 33 34 4.2. *FG-positive cells in the MPGs*

35 To assess the degree of neuronal survival, a retrograde tracing study using FG was  
36 carried out. Representative sections from four DMEM (Fig. 4A-D) and four MSC  
37 (Fig.4 E-H) animals are shown in Fig. 4A-H. However, we counted the number of  
38 FG-positive cells in the entire MPG in both groups. The mean number of FG-positive  
39 cells was  $58.3\pm 4.7$  (cells/MPG) in the MSC group as compared to the DMEM group  
40 ( $36.9\pm 3.8$  cells/animal) ( $P<0.01$ ) at 2 weeks after CN injury (Fig.4I). This indicated  
41 that there was 58.0 % higher number of FG detected neurons in the MSC treated rats.  
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#### 52 53 54 4.3. *Distribution of transplanted PKH26-labelled MSCs (PKH26-MSCs) in the* 55 *injured lesion site* 56 57 58 59 60

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3 The PKH26-MSCs (red) survived and distributed to the lesion sites. The  
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5 distribution of the transplanted PKH26-MSCs demonstrates a homing effect into the  
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7 MPGs (5-10 cells/slide) and lesioned CNs (10-20 cells/slide) in relation to the  
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9 neurofilament stained axons after systemic delivery (Fig. 5A, B). To determine if  
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11 autofluorescence of the MSCs was present at the wavelengths used to study PKH26  
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13 fluorescence, we examined MPGs and CNs of animals infused MSCs without labeling  
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15 PKH26. No red cells were observed in these lesions (Fig 5C, D).  
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#### 21 *4.4. Expression of GDNF and Neurturin mRNA after transplantation*

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23 To investigate the expression levels of GDNF and Neurturin mRNA in the MPGs,  
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25 we performed real-time quantitative RT-PCR. GDNF and Neurturin are neurotrophic  
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27 factors in MPG that are important for erection<sup>26, 27</sup>. Relative expression levels of  
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29 GDNF mRNA (Fig 6A) in MPG was significantly higher (3.6 fold) in the MSC group  
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31 (7.22±2.59) compared to the DMEM group (2.02±1.27) (P<0.01) and the relative  
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33 expression level of Neurturin mRNA (Fig 6B) in MPGs was also significantly higher  
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35 (2.4 fold) in the MSC group (4.03±1.84) compared to the DMEM group (1.67±0.65)  
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37 (P<0.05).  
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## 43 **5. Discussion**

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45 The primary objective of this study was to develop a new approach to prevent or  
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47 reduce post-operative ED with intravenous preload of MSCs in a CN injury rat model.  
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49 Intravenous preload of the MSC-infused group exhibited a significant preservation of  
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51 erectile function. Erectile functional in the MSC transplanted animals as assessed  
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53 using ICP/AP and ICP-AUC analysis was significantly preserved compared to the  
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55 control group. A retrograde tracing study using FG also demonstrated that the number  
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3 of FG-positive cells in the MPG was higher in the MSC group compared to the  
4 control group. MSCs labeled with PKH26 that is known to have a long half-life *in*  
5 *vivo*, were found in the both MPGs and injured CNs after the transplantation. We  
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7 observed significantly higher expression of GDNF and neurturin mRNA in MPG in  
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9 the MSC group by real-time quantitative RT-PCR. These results suggest that  
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11 intravenous preload (delivered before the surgical procedure) of MSCs rescues  
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13 erectile function by neuroprotection in a rat model of cavernous nerve injury. Given  
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15 that prostatectomy surgery unlike traumatic disorders is timed, the preparation and  
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17 pre-delivery of MSCs before surgery is practical. Thus, the therapeutic effects of  
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19 MSCs could have a more immediate beneficial effect.  
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25 Although the post-operative ED occurred in both groups, the MSC-preloaded  
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27 group displayed less ED shortly after CN injury (Fig. 2) and significant improvement  
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29 at 2 weeks (Fig. 3) evaluated with physiological analyses suggesting that the ED in  
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31 the MSC group gradually improved over time. Acute accumulation (less than 1 hour)  
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33 of the intravenously preloaded PKH-labelled MSCs observed in the MPG neurons and  
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35 injured CNs might provide neuroprotection. Fandel et al. reported that  
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37 intracavernously injected adipose-derived stem cells were detected in the MPG and  
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39 found the elevation of stromal cell-derived factor-1 (SDF-1) in the MPG<sup>18</sup>. SDF-1 is  
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41 a chemokine that has been implicated in cell recruitment (homing) after stem cell  
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43 infusion<sup>18</sup>. MSCs also have a tendency to home to damaged lesion sites and enhance  
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45 their therapeutic effectiveness with similar molecular mechanisms<sup>28</sup>.  
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49 Elevation of GDNF and neurturin mRNA in MPG was associated with the  
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51 preservation of the erectile function in the MSC group. GDNF and neurturin are two  
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53 of the neurotrophic factors acting in the MPG for erection<sup>26</sup>. Kato et al. demonstrated  
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55 that the recovery from ED after CN crush was facilitated when GDNF and neurturin  
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3 were administered into nerves with herpes simplex virus vector-mediated delivery<sup>20</sup>,  
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5 <sup>23</sup>. Another possible therapeutic mechanisms of intravenously transplanted MSCs in  
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7 various models of nervous diseases are not only differentiation to neuronal and/or  
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9 glial cells, but also secretion of neurotrophic factors which can provide for  
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11 neuroprotection, regeneration, recruitment of progenitors, facilitation of endogenous  
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13 neurotrophic secretion, induction of axonal sprouting, remyelination and modulation  
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15 of inflammatory/immune responses <sup>10, 29</sup>. Recent studies also suggested the  
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17 involvement of paracrine pathways. Paracrine action might be an important  
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19 mechanism for erectile function by which transplantation of MSCs facilitate  
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21 regeneration in corpus cavernosum after CN injury <sup>30, 31</sup>.  
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25 Preloaded MSCs could circulate in the blood stream and home to the injured  
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27 tissues such as MPGs and injured CNs when the insults occurred and the distributed  
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29 these cells could have therapeutic efficacy for post-operative ED. In experimental  
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31 erectile function, several studies using intracavernous injection of MSCs have been  
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33 reported therapeutic effects<sup>32</sup>. Yet, there are few studies using intravenous infusion of  
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35 MSCs for ED. Intravenous preloading of MSCs, which may home to MPG and  
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37 injured CNs after the CN injury, would be less invasive than intracavernous injection.  
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39 The other potential merit of intravenous infusion of the MSCs is that infused MSCs  
40  
41 accumulated not only in the injured CNs but also in the MPG providing significant  
42  
43 recovery of erectile function after CN injury. Given that intravenous infusion of  
44  
45 MSCs derived from bone marrow has been used in a number of clinical studies with  
46  
47 demonstrated safety the prospect of using MSCs as an adjunct therapy for damaged  
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49 nerve repairs should be considered. Functional improvement after intravenously  
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51 delivered MSCs has been reported in several clinical studies dealing with other  
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53 neurological diseases <sup>13, 15</sup>. Currently, clinical trials with direct injection of adipose-  
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3 derived MSCs for ED (ClinicalTrials.gov Identifier: NCT02087397; NCT02240823)  
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5 are listed and the intravenous infusion of bone marrow-derived MSCs for other  
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7 neurological diseases such as cerebral stroke, spinal cord injury are also ongoing<sup>13, 15</sup>.  
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10 Whether intravenous infusion of MSCs for prostate cancer promotes progression  
11 or not is still controversy. There are several papers evaluating in progression of  
12 experimental prostate cancer, most of them concluded MSC had no adverse effects on  
13 progression of prostate cancer, most of them concluded MSC had no adverse effects on  
14 progression of prostate cancer<sup>33, 34</sup>. We can also select the patients with low-risk by  
15 combined information of MRI, biopsy Gleason score, and preoperative PSA level  
16 before RP. Although we agree that the intravenous infusion of MSCs would be  
17 contraindicated in case of aggressive cancers<sup>35</sup>, twelve-year cancer specific survival  
18 was reported more than 95% for patients with low risk prostate cancer who received  
19 RP<sup>36</sup>. Therefore, intravenous infusion of MSCs before RP for these selected patients  
20 might be safe in terms of recurrence, progression and overall survival.  
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32 Our study has limitations. In this study, we focused on the primary change by  
33 assessment of the degree of neural survival to examine FG-positive cells in the MPG  
34 and found that the number of FG-positive cells in the MPG was higher in the MSC  
35 group compared to the control group indicating greater neuronal connectivity. We  
36 also performed the ICP/AP and ICP-AUC analysis to examine erectile function and  
37 found that post-operative ED was prohibited in the MSC group. Since we did not  
38 focus on the secondary change, we did not assess the expression of nNOS and eNOS  
39 in the cavernous nerves and in the penis, which play a major role in erection and are  
40 impaired after CN injury. Future studies will be performed to elucidate the molecular  
41 mechanism remains to be determined.  
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54 In summary our results indicate that intravenous preload of MSCs prevents or  
55 reduces post-operative ED in a CN injury model. Potential mechanisms include  
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3 distribution of the transplanted cells to the lesion area where they may provide  
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5 neuroprotection and prevent neuropraxia. It should be noted that we infused MSCs  
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7 before CN injury, suggesting that preload of MSCs can rescue erectile function..  
8  
9 Finally it may be possible to combine MSC therapy with other current penile  
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11 rehabilitation therapies to achieve maximal function.  
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18  
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**Figure legends****Figure 1**

A timeline for experiments is shown. Animals were first assessed by ICP/AP. They were randomized to two groups and preloaded (MSC and DMEM groups), then induced CN injury. The ICP/AP was measured at 1 hour and two weeks. Tissue sampling for histological analyses were performed at 1 hour and 2 weeks.

**Figure 2**

ICP response to electrostimulation of CNs at 1 hour. ICP along with AP (ICP/AP): (A) in the pre-injury, MSC group, (B) pre-injury, DMEM group, (C) post 1 hour, MSC group, (D) post 1 hour, DMEM group. Quantitative analyses of erectile function as evidence by ICP/AP (E) and ICP-AUC (F). ICP/AP and ICP-AUC at 1 hour after injury in the MSC group was significantly higher compared to the ICP/AP at 1 hour after injury in the DMEM group (\*  $P < 0.05$ ). The ICP/AP and ICP-AUC were significantly decreased one hour after injury in the both groups (\*\*  $P < 0.01$ ). Black bars under the x axis indicate 1min period of stimulation.

**Figure 3**

ICP response to electrostimulation of CNs at 2 weeks. ICP along with AP (ICP/AP): (A) MSC and (B) DMEM groups. Rats preloaded MSCs exhibited significant recovery of ICP/AP and ICP-AUC compared to the DMEM group. Quantitative analyses of erectile function as evidence by ICP/AP (C) and ICP-AUC (D). The ICP/AP and ICP-AUC at 2 weeks after injury in the MSC group was significantly higher compared to the DMEM group (\*\*  $P < 0.01$ , \*  $P < 0.05$ ). Black bars under the x axis indicate 1min period of stimulation.

**Figure 4**

Representative images of FG-positive cells in the MPGs from four animals per group 2 weeks after transplantation; (A-D) DMEM, (E-H) MSC groups. (I) The MSC group had more FG-positive cells in MPGs than DMEM group. \*\*  $p < 0.01$ . Scale bar = 250  $\mu\text{m}$ .

**Figure 5**

Transverse sections and immunohistochemical analysis of injured MPG (A) and CN (B) and intravenous preload of PKH26-MSCs. Demonstration of distributed infused PKH26-MSCs (red: white arrows) and stained with neurofilament (green). Endogenous cells stained with DAPI (blue). Note that we infused MSCs without labeling PKH26 and no red cells were observed in the MPGs (C) and CNs (D). Scale bar = 150  $\mu\text{m}$ .

**Figure 6**

mRNA expression of GDNF (A) and Neurturin (B) in MPGs after intravenous preload of MSCs at 1 hour. Relative expression level of GDNF and Neurturin mRNA in MPGs was significantly higher in the MSC group ( $P < 0.01, 0.05$ ).

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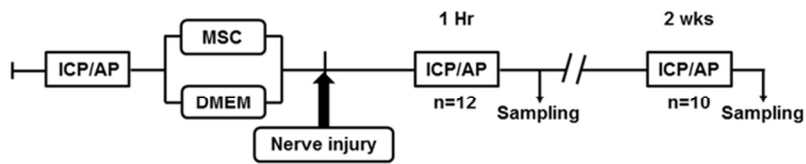
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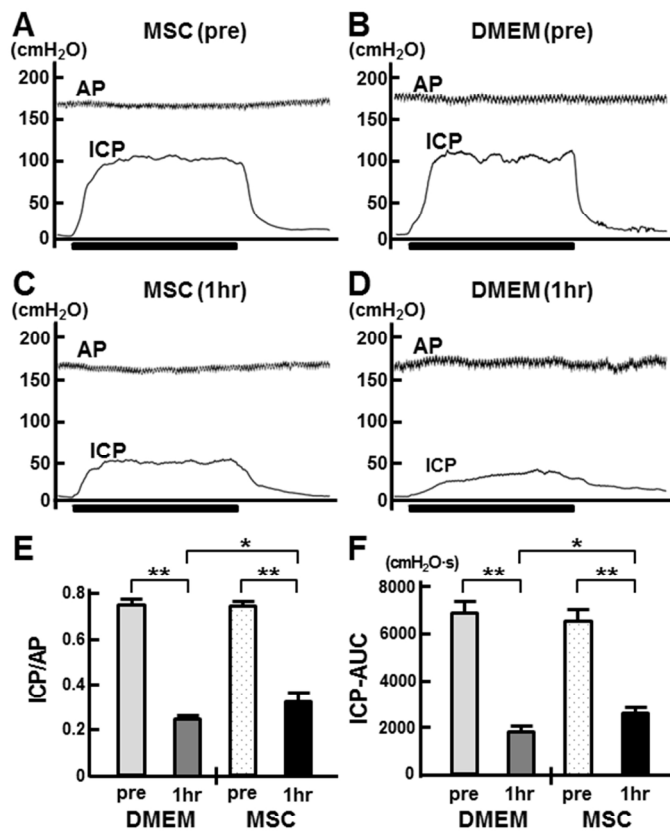


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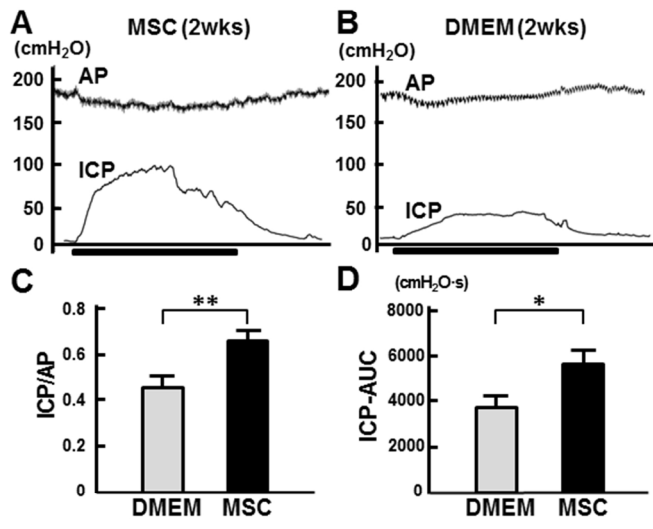
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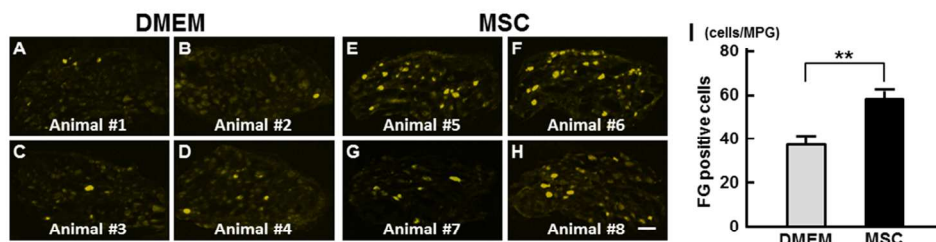
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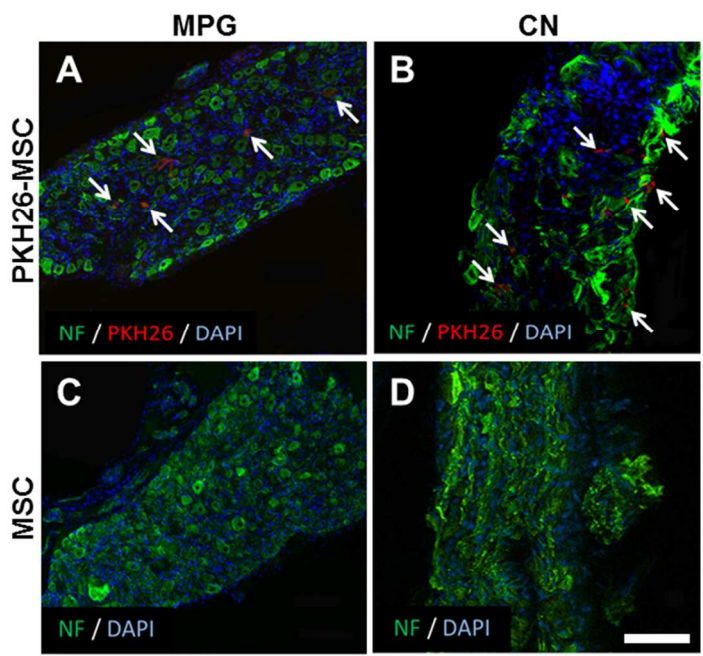
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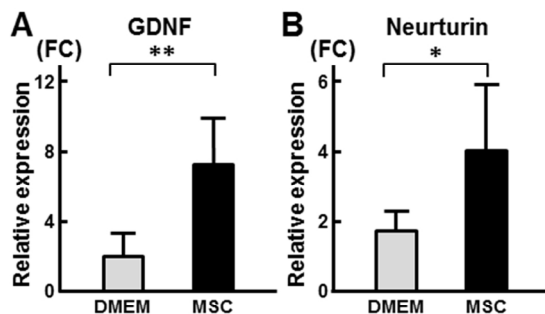
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