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Bone marrow–derived mesenchymal stromal cell therapy in a rat model of cavernous nerve injury: Preclinical study for approval

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Abstract

Background aims. Although clinical studies using stem cells to treat erectile dysfunction have been performed or are ongoing, there is little consensus on the optimal protocol. We aimed to develop a protocol optimizing human bone marrow–derived mesenchymal stromal cell (hBMSC) therapy in a rat model of cavernous nerve injury. *Methods.* We performed, in order, a dose-finding study, a toxicokinetic study of hBMSCs, and a study to determine the timing and number of cell injections. *Results.* From the dose-finding study, 1×10^6 cells were selected as the dose per hBMSC injection. From the toxicokinetic study, 14 days was selected as the interval between repeat treatments. In the final study, the ratio of maximal intracavernous pressure to mean arterial pressure was significantly lower in the control group than in the sham group (23.4% vs. 55.1%, $P < 0.001$). An immediate single injection of hBMSCs significantly improved erectile function compared with the control group (39.8%, $P = 0.035$), whereas a delayed single injection showed improvement with a marginal trend (38.1%, $P = 0.079$). All histomorphometric changes were significantly more improved in the immediate or delayed single injection groups than in the control group. Repeat treatments did not provide any benefit for the recovery of erectile function and histomorphometric changes. *Conclusions.* Intracavernous injection of 1×10^6 hBMSCs results in a recovery of penile erection and histomorphometric changes in a rat model of cavernous nerve injury, even when treatment was delayed until 4 weeks after cavernous nerve injury.

Key Words: *cavernous nerve, impotence, penile erection, prostatectomy, stem cell transplantation*

Introduction

Erectile dysfunction (ED) after radical prostatectomy (RP) typically results from injury to the cavernous nerve (CN) that runs along the posterolateral aspect of the prostate [1]. Most CN injuries are neuropraxic, which refers to a failure of nerve conduction in the absence of structural change [2]. As a result, decreased arterial flow and a chronic absence of erections induce long-term intracorporal hypoxia, resulting in apoptosis of corporal smooth muscle cells and deposition of collagen fibers [3,4]. An imbalance between the numbers of corporal smooth muscle cells and collagen fibers leads to veno-occlusive dysfunction and increased venous leak in affected patients [5]. Phosphodiesterase type 5 inhibitors are commonly used to treat ED after RP, but the efficacy of these drugs in this population would not be expected to be high as in the general population of ED patients [6].

Recent research approaches for ED after RP include the use of stem cell–based therapies. We also found that intracavernous injection of human bone marrow–derived mesenchymal stromal cells (hBMSCs) in a rat model of CN injury induces penile erection recovery and that periprostatic implantation of hBMSCs potentiates the recovery of erectile function by intracavernous injection via regeneration of neuronal nitric oxide synthase (nNOS)-containing nerve fibers [7]. Several human clinical studies that use various types of stem cells to treat ED have been

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performed or are ongoing [8–14]. However, there is little consensus on the protocol for optimizing stem cell–based therapies, especially for ED following RP. In our present study, we aimed to develop a protocol for optimizing hBMSC therapy in a rat model of CN injury. Our analysis consisted of three parts, a dose-finding study, a toxicokinetic study of hBMSCs and a study of the timing and number of cell injections.

Methods

Isolation and culture of hBMSCs

The study protocol was approved by the Institutional Review Board of Asan Medical Center, Seoul, Republic of Korea (2013-0158) and conformed to the tenets of the Declaration of Helsinki. All donors gave informed consent. Bone marrow was obtained from three healthy donors. All the manufacturing and product testing procedures for the generation of hBMSCs were performed under Good Manufacturing Practice conditions (Pharmicell Co. Ltd.). Approximately 10 mL of bone marrow was obtained from the posterior superior iliac crest of donor. Mononuclear cells were separated from the bone marrow by density gradient centrifugation (Ficoll-Paque, 1.077 g/L, Sigma) and washed with phosphate-buffered saline (PBS). Cells were resuspended in Dulbecco's Modified Eagle's Medium—low glucose (Gibco) containing 10% fetal bovine serum (catalog no. 10099, lot no. 1376155; Gibco) and 20 μg/mL gentamicin (Gibco) and plated at a density of 1.0–1.5 $\times$ 10^5 cells/cm^2 in 75 cm^2 or 175 cm^2 flasks. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2. After 5–7 d, the nonadherent cells were removed by replacing the medium and the adherent cells were cultured another 2–3 d. After reaching 70–80% confluence, the adherent cells were detached with trypsin containing ethylenediaminetetraacetic acid (Gibco) and replated at a density of 4–5 $\times$ 10^3 cells/cm^2 in 175 cm^2 flasks (catalog no. 159910, lot no. 7092806; Thermo Fisher Scientific Inc.). Cells were serially subcultured up to passage 5 for animal injection. On the day of injection, hBMSCs were harvested using trypsin, washed twice with PBS and once with Plasma Solution A Inj. (Multiple Electrolytes Injection, Type 1, USP; CJ HealthCare) and resuspended to a final concentration of 1–4 $\times$ 10^7 cells/mL in Plasma Solution A Inj. Criteria for release of hBMSCs for preclinical use included absence of microbial contamination (bacteria, fungus, mycoplasma or endotoxin), viability greater than 70% when assessed using a trypan blue exclusion assay and immune phenotyping by flow cytometric analysis proving expression of CD73 and CD105 surface molecules (>85%) and absence of CD14, CD34 and CD45 (<3%).

Animal care

All aspects of animal care and treatment and the surgical procedures used conformed to the eighth edition of the *Guide for the Care and Use of Laboratory Animals* published in 2011. The protocols for animal experimentation were approved by the Institutional Animal Care and Use Committee of Asan Medical Center (2012-12-252). One hundred forty 8-week-old male Sprague-Dawley rats were purchased from Orient Bio and housed for 1 week for acclimatization. During the experiments, all rats were maintained under a 12 h:12 h light/dark cycle (lights on at 8:00 AM, lights off at 8 PM), a temperature of 22 ± 2°C, and a humidity of 50–55% with ad libitum access to food and water.

Dose-finding study design

At 9 weeks of age, 50 rats were randomly divided into five equal groups (10 animals per group): sham group, control group, low-dose group (5 $\times$ 10^5 hBMSCs), moderate-dose group (1 $\times$ 10^6 hBMSCs) and high-dose group (2 $\times$ 10^6 hBMSCs). After randomization, laparotomy was performed in the sham group, whereas bilateral CN injury was induced in the other four groups. At the same time, hBMSCs were injected intracavernously in the low-, moderate- and high-dose groups. After 4 weeks, all rats underwent erectile function evaluation. They were then sacrificed, and tissues were harvested for histological examination.

Toxicokinetic study of hBMSCs

At 9 weeks of age, 30 rats were randomly divided into a sham group and an ED group (15 animals per group). After randomization, laparotomy was performed in the sham group, whereas bilateral CN injury was induced in the ED group. At the same time, the number of hBMSCs selected by dose-finding study was injected intracavernously in both groups. For toxicokinetic study, rats were sacrificed at various time points after intracavernous injections of hBMSCs (1, 3, 7, 14 and 28 d). Tissues and fluids were then harvested including blood, bone marrow, brain, heart, kidney, liver, lung, major pelvic ganglia (MPG), pancreas, penis, prostate, spinal cord, spleen, skeletal muscle, testis and thymus.

The collected tissues were washed with PBS and stored at −80°C until genomic DNA was extracted. Genomic DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega Corporation). Positive control genomic DNA was isolated from hBMSCs, and negative genomic DNA was isolated from the identical tissues of non-transplanted animals. To check the quality of the isolated genomic DNA, we amplified a 433-bp fragment using primers for the murine-specific c-mos oncogene in a PTC-100 thermal