Available online at www.sciencedirect.com

ScienceDirect

Journal of the Chinese Medical Association xx (2017) 1–9

www.jcma-online.com

Original Article

Mesenchymal stem cells and their conditioned medium can enhance the repair of uterine defects in a rat model

Chi-Hong Ho [a,b,e], Chen-Wei Lan [c], Chen-Yi Liao [c], Shih-Chieh Hung [d], Hsin-Yang Li [a,c,e], Yen-Jen Sung [c,*]

[a] *Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, Taipei, Taiwan, ROC*
[b] *Institute of Physiology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, ROC*
[c] *Institute of Anatomy and Cell Biology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, ROC*
[d] *Institute of Clinical Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan, ROC*
[e] *Division of Obstetrics and Gynecology, Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan, ROC*

Received January 19, 2017; accepted March 18, 2017

Abstract

Background: Our aim was to examine the roles of mesenchymal stem cell (MSC) transplantation in the repair of large uterine defects.
Methods: Uterine defects were created in both uterine horns of female rats by a punch instrument, and bone marrow-derived MSCs, MSC-conditioned medium (MSC-CM) or vehicle were injected into the myometrium around the defect. The rate of uterine defect repair was monitored on day 2 and 4 after operation. Cytokine array of MSC-CM was performed, followed by neutralizing antibody experiments to clarify the exact cytokine participating in the MSC-CM-enhanced wound repair.
Results: Transplantation of MSCs, but not myometrial cells, significantly enhanced uterine defect repair. The transplanted MSCs were detected in the uterine horn with no signs of rejection on day 4 after transplantation, when the MSC-transplanted uterine wound was nearly healed. Moreover, uterine defect repair was also accelerated by injection of MSC-CM, indicating the paracrine effects of MSCs on uterine wound healing. Cytokine array analysis further revealed that MSC-CM contained abundant cytokines and chemokines, among which high levels of interleukin-6 (IL-6) were found. Additionally, antibodies against IL-6 were shown to block MSC-CM-enhanced uterine defect repair.
Conclusion: This study demonstrated that transplantation of MSCs could enhance uterine defect repair by paracrine effects involving IL-6, which are findings that may be applied to facilitate uterine wound healing in the removal of huge intramural masses.

Keywords: Mesenchymal stem cells; Paracrine effects; Transplantation; Uterine defect repair; Uterine surgery

1. Introduction

Extensive uterine surgery, such as removal of huge uterine myomas or adenomyomas, can occasionally result in uterine rupture during subsequent pregnancy, particularly when the surgery is performed laparoscopically.[1–8] The risks of uterine rupture after myomectomy depend on the size and location of the myomas, whether the endometrial cavity is entered, and whether the myometrial defect is adequately repaired.[9] While the incidence of uterine rupture in pregnancies following laparoscopic myomectomy is not high (0.26% reported in one series), uterine rupture can lead to catastrophic maternal death as well as fetal mortality and morbidity.[1,10] To minimize post-myomectomy complications, some adjuvant treatments have been proposed for the repair of myometrial defects. For example, fibrin glue has been used in the repair of uterine

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Yen-Jen Sung, Institute of Anatomy and Cell Biology, School of Medicine, National Yang-Ming University, 155, Section 2, Li-Nong Street, Taipei 112, Taiwan, ROC.
E-mail address: yjsung@ym.edu.tw (Y.-J. Sung).

http://dx.doi.org/10.1016/j.jcma.2017.03.013

defects; however, no benefit has been found.[11] Therefore, a new approach to facilitate uterine defect repair following extensive uterine surgery is warranted.

Mesenchymal stem cells (MSCs) are multipotent progenitors capable of differentiating into various lineages, including osteoblasts, chondrocytes, and neurons.[12–14] Sources of MSCs include bone marrow (BM), Wharton's jelly of umbilical cord, amniotic fluid, and decidua.[13,15–17] One of the advantages of BM-derived MSCs is that they can be of the patient's own origin, thereby avoiding immune rejection after transplantation. Transplantation of stem cells into recipients via local delivery or systemic injection may satisfy clinical application for tissue repair. Current therapeutic applications of MSCs include myocardial infarction,[18,19] stroke,[20] osteogenesis imperfect,[21] and tendon defect.[22]

Although transplanted MSCs *per se* can be induced to give rise to various differentiated cell types under both *in vivo* and *in vitro* conditions, mechanisms that transplanted MSCs may "drive" adjacent cells to proliferate and differentiate have been discussed. Prior studies have demonstrated that cardiac transfer of MSCs could favorably repair infarcted myocardium and nearly normalize cardiac performance[23]; however, the fact that functional improvement was observed as early as within 72 h has raised the question as to whether such early effects could be attributed to the myocardial differentiation from the transplanted cells.[24] It has been demonstrated that the enhanced tissue repair and functional improvement after MSC transplantation into infarcted hearts are mediated by paracrine action of MSCs.[24,25] Paracrine signaling molecules, such as cytokines and growth factors, released by transferred MSCs could stimulate resident stem cells near the injured tissue to repair the defect.[26]

The purpose of this study was to examine the roles of MSC transplantation in the repair of uterine defects. Uterine defects were made in both uterine horns of female rats by a punch instrument, followed by injection of BM-derived MSCs or vehicle (phosphate-buffered saline, PBS) into the myometrium around the defect. Thereafter, the rate of uterine defect repair was monitored. Furthermore, the effect of MSC-conditioned medium (MSC-CM) on uterine defect repair was studied to determine if paracrine signaling mediated the effects of transplanted MSCs on uterine defect healing.

2. Methods

2.1. Mesenchymal stem cell culture

Primary MSCs from BM of three normal human volunteers were obtained from the Tulane Center for Distribution of Adult Stem Cells (New Orleans, LA, USA) and prepared as previously described.[27] The cells were seeded at 100 cells per cm^2 and grown in a complete culture medium consisting of α-minimal essential medium (Invitrogen, Carlsbad, CA, USA) supplemented with 16.6% fetal bovine serum (Hyclone, Logan, UT, USA), 100 units/mL penicillin (Invitrogen), 100 μg/mL streptomycin (Invitrogen), and 2 mm L-glutamine (Invitrogen). The growth medium was changed every two days, and cells were subcultured by trypsinization (trypsin-EDTA solution; Invitrogen) when they reached approximately 80% confluence. Flow cytometry analysis for surface marker detection was performed as previously described.[13] Osteogenic, adipogenic and chondrogenic differentiation of MSCs was induced according to the protocols previously described.[13]

2.2. Preparation of MSC-conditioned medium

MSCs were seeded at 10,000 cells per cm^2 and incubated in a complete culture medium for 1 day. The attached cells were washed three times with PBS, and the medium was replaced with serum-free basal medium and incubated for 48 h. The MSC-CM was collected, centrifuged at 1500× *g* for 10 min to remove cell debris, and further concentrated 50× by ultrafiltration using centrifugal filter units with 5 kDa cut-off (Millipore, Billerica, MA, USA) following the manufacturer's instructions.

2.3. Myometrial cell culture

All animal experiments were approved by the Institutional Animal Care and Use Committee of National Yang-Ming University. Uterine horns were isolated from 8 week-old female Sprague-Dawley rats (National Yang-Ming University, Taipei, Taiwan), and the endometrial tissue was scraped off. This was followed by enzymatic digestion of the remaining myometrial tissue by incubating in HBSS (Invitrogen) containing collagenase (0.1%; Sigma, St Louis, MO), DNAse I (0.02%; Sigma), protease and trypsin (0.01%; Sigma) for 10 min at 37 °C with shaking. After discarding the supernatant, the fresh enzyme solution was replaced and incubated for another 10 min, when the tissue pieces were gently broken up by drawing the mixture slowly through a large-bore siliconized pipette and this procedure was continued for 30 s every 3 min. Then the mixture was centrifuged at 20*g* for 5 min and supernatant was removed and saved, followed by the addition of fresh enzyme solution to the remaining pieces. The above procedures were repeated three times, and the combined supernatant solution containing myometrial cells was filtered through nylon mesh and centrifuged at 430*g* for 10 min. The cell pellet was resuspended in 1:1 DMEM:F12 nutrient medium (Invitrogen) containing 10% FBS, 1% antibiotics and sodium bicarbonate, and plated in 24-well plates. More than 95% of the cells in culture were identified as smooth muscle cells by the detection of smooth muscle actin (SMA) by immunocytochemistry (Abcam, Cambridge, MA, USA; Fig. 4A).

2.4. Cytokine array

Membranes from a human protein cytokine array kit (Proteome Profiler™ Array; R&D, Minneapolis, MN, USA) were used to assay relative levels of 36 cytokines/chemokines in the 50× concentrated MSC-conditioned medium according to the manufacturer's instructions.

Please cite this article in press as: Ho C-H, et al., Mesenchymal stem cells and their conditioned medium can enhance the repair of uterine defects in a rat model, Journal of the Chinese Medical Association (2017), http://dx.doi.org/10.1016/j.jcma.2017.03.013