Mesenchymal stem-cell therapy alleviates interstitial cystitis by activating Wnt signaling pathway

Miho Song1, Junsoo Park1, Won Hee Park1, Kun Suk Kim1, Sungchan Park1, Ji-Yeon Han1, Myung-Soo Choo1

1Department of Urology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; 2Department of Urology, Pusan National University Yangsan Hospital, Yangsan, Korea; 3Department of Pathology, Pusan National University Yangsan Hospital, Yangsan, Korea

Introduction

Interstitial cystitis (IC) is a devastating disease with no reliable treatment, but the appropriate applications of stem cell therapy remain unproven.

Purpose

This study evaluated the therapeutic potency of using human umbilical cord-blood derived mesenchymal stem cells (UCB-MSC) to treat IC in a rat model and investigate its responsible molecular mechanism.

Materials and Methods

• Design, setting, and participants:
  - IC was induced in 10-weeks-old female Sprague-Dawley rats via the installation of 0.1 M HCl or PBS (sham).
  - After 1 week, human UCB-MSC (IC+MSC) or PBS (IC) was directly injected into the submucosal layer of the bladder.

• Outcome measurements and statistical analysis:
  - Cystometric parameters, histological examination, immunostaining, and gene expression were measured at 1 week after intervention.

Results

Figure 1. UCB-MSC injection improved voiding function in an IC bladder. (A) Representative cystometry results and (B) contraction intervals in the indicated animal groups at 1 week after the injection of mesenchymal stem cells (MSCs). Data are represented as dot plot with the mean ± SEM (red line). p < 0.05 compared with sham-operated (sham) group (arrow). Scale bar = 20 μm.

Figure 2. UCB-MSC therapy ameliorated histological abnormalities in IC bladder. (A) H&E staining in the indicated bladder tissues (magnification ×200). Nuclei were stained with Mayer’s hematoxylin. Arrows indicate severe inflammation, (B) magnification ×400, and vimentin staining (C; magnification ×200) were used to evaluate the infiltration of master cells and the integrity of the urothelium, respectively. Arrows indicate the broken urothelium.

Figure 3. Engraftment of injected UCB-MSCs. Fluorescent immunohistochemical detection of the PKH26-labeled UCB-MSCs (red), which colocalized with (A) cytokeratin+ urothelium and (B) vimentin+ stromal tissue (green) in the IC bladder tissues at 1 week after stem cell injection. Nuclei were stained with DAPI (blue). The dotted line indicates the margin between the urothelium and stromal tissues. The left and right panel images are magnified ×200 and ×400, respectively.

Figure 4. Activation of an Shh-Wnt-EGF signaling cascade by engrafted UCB-MSCs. RT-PCR analysis of (A) Shh, (B) Wnt, and (C) downstream growth factors in the indicated bladder tissues. Expression is represented as %GAPDH (as determined using ≥ 5 independent experiments) and is shown as dot plot with the mean ± SEM (red line). p < 0.05, **p < 0.01 compared with sham-operated rats)

Figure 5. Wnt-EGF signaling activity regulates the therapeutic effects of UCB-MSC in IC bladder. (A) Contraction intervals of the IC bladders that were injected with UCB-MSC in the absence or presence of indomethacin (Indo, Wnt blocker) or Cal fluid (cell, EGF-R blocker). Data are shown as the mean ± SEM (n = 4; p < 0.05, *p < 0.01 in comparison with IC bladders). (B) Fluorescent immunohistochemical detection of cytokeratin+ epithelium (green) at 1 week after the injection of PKH26-labeled UCB-MSC (red) in the absence or presence of inhibitors. Nuclei were stained with DAPI (blue). The region characterized with the broken urothelium (box in left panel image; magnification ×200, scale bar = 50 μm) is shown in the right panel at higher magnification ×400, scale bar = 20 μm.

Figure 6. Effects of Wnt-EGF signaling activity on the gene expression of the Shh-Wnt-EGF cascade components. RT-PCR analysis of the (A) Shh, (B) Wnt, and (C) downstream growth factors in IC bladder tissues that were injected with UCB-MSCs. Expression levels in the indicated bladder tissues are represented as %GAPDH (as determined using ≥ 5 independent experiments) and are shown as dot plot with mean ± SEM (p < 0.05, **p < 0.01 compared with sham-operated rats).

Figure 7. Explanation of how UCB-MSC therapy is curative for IC bladder. The loss of urothelial integrity caused by multiple bladder insults (dotted arrow) leads to pathophysiologies characteristic of IC patients, including epithelial denudation and the abnormal increase in inflammation, neural, and angiogenesis. Injected UCB-MSCs in a paracrine manner stimulate the regenerative capability of endogenous stem cells (solid arrow). In addition, the engrafted UCB-MSCs directly differentiate into epithelial cells in the bladder tissues.

Conclusions

This is the first report that provides an experimental evidence of the therapeutic effects and molecular mechanisms of MSC therapy to IC using an orthodox rat animal model. Our findings not only provide the basis for clinical trials, but also advance our understanding of IC pathophysiology.