509

Kim A[1], Ryu C[1], Kyung Y S[1], Han J[2], Shin D[3], Chung J Y[4], Ahn T Y[1], Choo M[1]
1. *Department of Urology, Asan Medical Center,* ***2.*** *Pusan National University Yangsan Hosptal,* ***3.*** *Department of Biomedical science, Asan Medical Center,,* ***4.*** *Inje University Sanggye Paik Hospital*

MESENCHYMAL STEM CELLS IMPROVED BLADDER FUNCTION AND HISTOPATHOLOGICAL FEATURES IN PROTAMINE SULFATE-LIPOPOLYSACCHARIDE INDUCED INTERSTITIAL CYSTITIS IN RAT BLADDER

Hypothesis / aims of study
The present study developed the most proper interstitial cystitis (IC) rat model which represented important features of human IC bladder, such as irregular and frequent micturition, denudated urothelium and increased inflammation and mast cell infiltration. Moreover we evaluated the therapeutic effect of Mesenchymal stem cells (MSCs) in employing the IC rat model.

Study design, materials and methods
Female 10-week-old Sprague-Dawley rats were given protamine sulfate (PS, 10mg) to bladder through PE-50 catheter in order to make denudation in urothelium. After 45 minutes the bladders were emptied, washed with buffer solution and then given a second treatment with lipopolysaccharide (LPS, 750ug) for 30 minutes in order to induce inflammation. Weekly instillations of PS/LPS following this regimen over a period 5 weeks were used to induce a longer-lasting and possibly chronic injury to the urothelium. One week after final administration of PS/LPS, one million of MSCs (n=10, IC+MSCs) or PBS (n=10, IC) were injected into the submucosal layer of the anterior wall and dome of the bladder. The therapeutic effect of MSCs was examined by awake cystometry, histological and gene expression analysis after 1 week of MSCs injection.

Results
IC Rat group exhibited irregular voiding frequency, decreased inter-contraction intervals, micturition volume and increased residual volume. A single injection of MSCs significantly improved most of voiding parameters by increased the inter-contraction interval, increased micturition volume and decreased residual volume. The bladder of IC group rats were characterized with severe denudated urothelium with inflammation, increased mast cell infiltration and paralleled with down-regulation of Wnt-8a, Wnt-8b and Wnt-1. Of note, a single injection of MSCs significantly not only improved the bladder voiding parameters but also reversed the histological and gene expression alternations characteristic for IC bladder.

Interpretation of results
We demonstrate that MSC therapy have therapeutic effect to repair voiding function and regenerate denudated urothelium and stabilize mast cell infiltration in the most proper IC rat model. Through these findings, we propose MSC therapy as a new therapeutic approach to treat uncontrolled painful bladder disorder.

Concluding message
MSC therapy can improve the uncontrolled painful bladder disease.

Figure 1.Schematic diagram of study design.
Ten female Sprague-Dawley rats were used in each group. Sham group had no MSC or PBS injection. Experimental control (IC group) were instilled with Protamine Sulfate (PS, 10 mg/kg) + Lipopolysaccharide (LPS, 750 μg/kg) once per week for five weeks. For interventions, a single administration of MSCs at the dose of $1x10^6$ into the submucosal layer of the bladder (IC+M-MSC group) at the indicated schedules.

Figure 2. Administration of MSCs improves the voiding function of rats with KC.
(A)Representative cystometry results of the indicated groups. (B) micturition intervals, (C) micturition pressure, (D) Residual volume, (E) micturition volume were quantified from voiding pattern analysis from at least 10 independent experiments. All data are represented as the mean±SEM. **p*<0.05, ***p*<0.01, ****p*<0.001 when the groups were compared by one-way analysis of variance with Bonferroni post-test.

Figure 3. Histological analysis of the beneficial effect of MSCs on PS/LPS-induced bladder injuries.

- **A.** H&E staining in the indicated bladder tissues (magnification ×200). Nuclei were stained with Mayer's hematoxylin. The upper and lower images were magnification x 40
  (scale bar:400μm) and x 100 (scale bar:200μm), respectively. LPS+PS: Lipopolysaccharide + protamine sulfate, MSC (Mesenchymal Stem Cell)
- **B.** The infiltrated mast-cell staining in bladder tissues were stained with Toluidine blue (red circles). The upper and lower images were magnification x 40 (scale bar: 400μm) and x 100 (scale bar: 200μm), respectively.

Figure 4. RQ-PCR analysis
The analysis showed inflammation related genes in the indicated bladder tissues. The expression level is represented as %*Gapdh* (as determined using ≥ 5 independent experiments) and is shown as the mean±SEM. ns; non-significant.

Disclosures
Funding: none **Clinical Trial:** No **Subjects:** ANIMAL **Species:** rat **Ethics Committee:** Asan medical center