Improved cytometric parameters using transplantation of mesenchymal stem cell into bladder in rats with spinal cord injury

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Hypothesis / aims of study
• In spinalized rats, inefficient bladder function might lead to complete deterioration of bladder function, infections and other lower urinary tract complications.
• The present study was performed to investigate human mesenchymal stem cells (B10) directly transplanted to the bladder wall are capable of inhibiting collagen deposition and improve cystometric parameters in SCI rats.

Study design, materials and methods
• B10 were labeled with fluorescent silica magnetic nanoparticles (MNP) contained rhodamine B isothiocyanate (RITC) conjugated to terminal silanol groups. Forty 6-week old female Sprague-Dawley rats were divided into 4 groups (group 1: control, group 2: sham operation, group 3: SCI, group 4: SCI rats receiving B10).
• For SCI model, contusion was performed on the thoracic spinal cord very severe intensity weight drop. Four weeks after the onset of SCI, B10 were injected into the bladder wall.
• Locomotor behavioral tests were performed using rotarod, Basso–Beattie–Bresnahan (BBB) test and voiding response was assessed at 4 weeks after transplantation and bladder was harvested. Nissl staining of spinal cord sections was performed.

Results

Figure 1. Functional test of SCI model after B10 transplantation. A: maintenance time on the rotarod, B: BBB Open-field locomotor scores for Sham, Injury. Injury+B10 groups tested at 4 weeks after transplantation.

Figure 2. In vivo MR imaging of MNP-labeled B10 cells into the bladder of SCI model of rats. A: Schematic drawing of bladder, urethra and transplanted HMSCs. B-D) Confirmation of transplanted MNP-labeled B10. B: The area of hypointense signal intensities after 4 weeks post-transplantation in MR imaging. C: In vitro immunostaining of fluorescent MNP - labeled B10. The abundant red rhodamine B isothiocyanate dots in the cytoplasm of the cells. D: B10 cells positive for human mitochondria antigen were found at the B10 transplanted bladder indicating that MNP-labeled B10 cells present in the bladder at 4 weeks post-transplantation. MR = magnetic resonance; MNP = magnetic nanoparticles; B = bladder wall; M = injected HMSC; U = urethra. Scale bar indicates C, 20μm; D, 50 μm.

Figure 3. Changes in weight of body (A) and bladder (B) after B10 transplantation. There was no significant difference of body weight between groups. The group with SCI showed increased bladder weight compared with the sham operation group (p<0.05). B10 cell transplantation showed decreased bladder weight as compared with the SCI group (p<0.05). B10-mesenchymal stem cells, Normal = control; SCI = rats with spinal cord injury; SCI+B10 = B10 transplantation rats with spinal cord injury.

Figure 4. Nissl staining of lesions spinal cord after SCI. Spinal cords of rat were isolated at 2 (A) and 8 weeks (B) after SCI. Nissl staining was performed in coronal sections of spinal cord to examine the regeneration of injured CNS. There were no the regenerated neurons around lesions from all slides and it means the spinal cord is not regenerated.

Figure 5. Histological change of collagen deposition after B10 HMSCs transplantation. Collagen deposition increased in the group with SCI and decreased after transplantation of B10 HMSCs. (A) Masson’s trichrome staining, A(x400), B(x400): Control, C(x100), D(x400): Sham, E(x100), F(x400): SCI, G(x100), H(x400): SCI+B10 HMSCs.

Figure 6. Differentiation of B10 HMSCs into smooth muscle (A-D). A. In transplanted bladder sections, B10 cells expressed immunoreactivity against human mitochondria. B. In transplanted bladder sections, B10 cells expressed immunoreactivity against SMA. C. Magnified view showed clear merged cells expressing yellow fluorescence were SMA positive cells that were derived from B10 cells. D. Magnified view showed clear merged cells expressing yellow fluorescence. B10 = human mesenchymal stem cells; SMS = smooth muscle actin. Scale bar indicates C, 50 μm; D, 20 μm.

Figure 7. Change of collagen deposition after B10 transplantation. The group with SCI showed increased percent collagen area, which decreased after transplantation of B10. B10 = human mesenchymal stem cells, Normal = control; SCI = rats with spinal cord injury; SCI+B10 = B10 transplantation rats with spinal cord injury.

Figure 8. Improvement of bladder function after transplantation of B10. A-D: CMG, A: control; B: Sham; C: ICI decreased in SCI rats; D: ICI recovered in SCI rats+ B10 transplantation. E-F: Analysis of CMG. E: The SCI rats showed decreased ICI compared with the sham operation group, which was reversed after transplantation of B10 HMSCs (p<0.05). F: The SCI rats showed increased MVP compared with the sham operation group, which was reversed after transplantation of B10 HMSCs (p<0.05).

G: There was no difference of PT between groups. B10 = human mesenchymal stem cells; CMG = cystometry; ICI = intercontraction interval; MVP = maximal voiding pressure; PT = threshold pressure; Normal = control; SCI = rats with spinal cord injury; SCI+B10 = B10 transplantation rats with spinal cord injury.

Interpretation of results

Transplantation of B10 to the bladder wall showed differentiation into smooth muscle cells, a lower collagen deposition and improved dysfunction of the bladder in the rat SCI model.

Concluding message

Transplantation of mesenchymal stem cells to the bladder wall could be a novel therapeutic strategy against bladder dysfunction in patients with SCI.