

MESENCHYMAL STEM CELLS FACILITATE PUDENDAL NERVE RECOVERY FROM SIMULATED CHILDBIRTH INJURY

Hypothesis / aims of study

The pudendal nerve innervates the external urethral sphincter (EUS) and can be injured during vaginal delivery, resulting in stress urinary incontinence. The EUS can also be injured via crush or ischemic injury during the 2nd stage of labor. The pudendal nerve recovers more slowly when the EUS is simultaneously injured, as can occur during childbirth [1]. Since bone marrow-derived mesenchymal stem cells can improve growth and myelination of regenerating axons [2], we hypothesized that intravenous (IV) bone marrow-derived mesenchymal stem cells (MSCs) would facilitate functional recovery after a simulated childbirth injury consisting of pudendal nerve crush (PNC) and vaginal distension (VD).

Study design, materials and methods

Twenty one age-matched virgin female Sprague-Dawley rats were divided into 3 groups. In the first group rats underwent VD & PNC and received saline IV as a sham treatment one hour after injury. In the 2nd group, rats underwent VD & PNC and received 2 million GFP-labeled MSC in saline IV one hour after injury. In the 3rd group, rats underwent sham VD & PNC and received saline IV one hour after injury. PNC was performed by crushing the pudendal nerve in the ischiorectal fossa using a Castroviejo needle holder 2 consecutive times, 30 seconds each time. Prior to VD the vagina was dilated under anesthesia by sequentially inserting increasing sized (26 - 32) Otis Bougie a Boule lubricated urethral dilators. A modified 10Fr Foley catheter was then inserted into the vagina and the balloon was inflated to 3 ml for 4 hours. Sham VD consisted of vaginal accommodation and catheter insertion for 4 hours without balloon inflation. Sham PNC consisted of gently opening the ischiorectal fossa without damaging the nerve. Leak point pressure (LPP), external urethral sphincter (EUS) electromyography (EMG), and pudendal nerve sensory branch potential (PNSBP) were tested 21 days after injury and treatment.

LPP and EUS EMG. Rats were anesthetized with urethane intraperitoneally and the urethra was exposed by opening the pubic symphysis with kaja scissors. Bipolar parallel platinum electrodes were placed on the outside of the mid-urethra at the location of the EUS and connected to an amplifier and electrophysiological recording system. A 2nd set of bipolar parallel platinum electrodes were placed on the sensory branch of the pudendal nerve. A polyethylene catheter (PE-50) was inserted into the bladder via the bladder dome, and connected to both a pressure transducer and syringe pump. Bladder pressure was referenced to air pressure at the level of the bladder. Bladder pressure, EUS EMG, and PNSBP were recorded while the bladder was filled with saline (5ml/hr). LPP testing was performed by increasing the intravesical pressure when the bladder was approximately half full by gradually pressing a cotton swab on the bladder until leakage occurred. At the moment of leakage, the cotton swab and the external pressure were rapidly removed. Values of bladder pressure just prior to LPP testing (tonic activity) and at the peak pressure (peak value) of LPP testing were determined. The urethral meatus and clitoris were gently brushed to generate an increased firing response in the PNSBP. Quantitative assessment of EUS EMG and PNSBP signals was performed by determining the mean rectified amplitude and the mean motor unit firing rate. For EUS EMG, these were calculated during tonic activity and at the pressure peak during LPP testing. For PNSBP, these were calculated during tonic activity and during brushing of the clitoris.

Data Analysis. Mean values of each of the quantitative variables were calculated for each animal and were used to calculate a mean and standard error of the mean for each group. One-way ANOVA followed by a Student-Newman-Keuls post hoc test was used to compare the quantitative variables. $P < 0.05$ was used to indicate a statistically significant difference between groups in all cases. Data is presented as mean \pm standard error of the mean (SEM).

Results

Peak pressure during LPP testing was significantly reduced after VD & PNC followed by sham treatment (27.8 ± 1.6 cm H₂O) compared to sham injured animals (37.5 ± 2.8 cm H₂O). In contrast, LPP was partially restored with MSC treatment after VD & PNC (32.6 ± 2.6 cm H₂O) and was not significantly different from either of the other groups. Baseline bladder pressure was unchanged by VD & PNC or by treatment with MSCs. EUS EMG amplitude and frequency both at baseline and during LPP testing were not significantly different between the 3 groups.

Tonic PNSBP activity (as measure by amplitude and firing rate) was unchanged by VD & PNC or by treatment with MSCs. Peak amplitude of PNSBP during clitoral brushing in sham injured rats (11.6 ± 0.7 μ V) was significantly greater than both of the VD & PNC groups, whether treated with saline (8.4 ± 0.3 μ V) or MSCs (9.4 ± 0.5 μ V). In contrast, peak firing rate of PNSBP during clitoral brushing was significantly decreased in sham-treated VD & PNC rats (20.8 ± 8.4 Hz) compared to both sham-injured rats (128.2 ± 7.7 Hz) and MSC-treated VD & PNC rats (73.1 ± 10.1 Hz). There was also a significant difference in peak firing rate between MSC-treated VD & PNC rats and sham-injured rats. Tonic firing rate of PNSBP was significantly greater in sham-injured rats (73.6 ± 14.0 Hz) than in either sham-treated VD & PNC rats (10.8 ± 6.0 Hz) or MSC-treated VD & PNC rats (35.6 ± 12.6 Hz). Although tonic firing rate was greater in the MSC-treated VD & PNC group than the sham treated VD & PNC group, this difference was not significantly different. However, when tonic PNSBP firing rate was subtracted from peak PNSBP firing rate to obtain a measure of the response of the pudendal nerve sensory branch to a stimulus, the sham treated VD & PNC group (10.0 ± 2.8 Hz) responded significantly less than either the sham-injured group (54.6 ± 10.5 Hz) or the MSC-treated VD& PNC group (37.5 ± 5.8 Hz).

Interpretation of results

The EUS EMG results indicate that the EUS recovers within 21 days after VD & PNC even when sham treated. At least some of the other contributors to continence, such as urethral smooth muscle and urethral mucosa, were not fully recovered by this time point since LPP in sham-treated VD & PNC animals was significantly decreased compared to sham-injured animals. MSCs partially facilitated recovery of continence, such that LPP in this group was not significantly decreased compared to the sham-injured group. Although amplitude of PNSBP was not improved by treatment with MSCs after VD & PNC, both peak firing rate and increase in firing rate of the PNSBP were significantly increased compared to the sham treated group. This suggests that the MSCs preserve neurological function and/or facilitate neuroregeneration and recovery of function of the sensory branch of the pudendal nerve. This may account in part for the improvement in LPP with MSC treatment, possibly via a reflex mechanism.

Concluding message

MSCs may represent a potential therapy to facilitate recovery after childbirth and prevent the sequelae of events that lead to stress urinary incontinence. Patients with postpartum incontinence may represent an initial population for therapy since postpartum incontinence is highly correlated with later development of stress urinary incontinence, even if it resolves initially. Since MSCs are thought to act via a paracrine mechanism [3], it is possible that, with further research to determine the mechanism of action of these cells, a noncellular therapy could be developed to deliver compounds similar to those being secreted by the MSCs.

References

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Disclosures

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