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BRAIN-DERIVED NEUROTROPHIC FACTOR IS AN IMPORTANT, BUT NOT THE ONLY THERAPEUTIC FACTOR IN A NOVEL STEM CELL SECRETION TREATMENT FOR STRESS URINARY INCONTINENCE

Hypothesis / aims of study

Current treatments for stress urinary incontinence (SUI) do not repair the neuromuscular continence mechanism that can be damaged in childbirth. Stem cells, including mesenchymal stem cells (MSCs) and their secretions, including brain-derived neurotrophic factor (BDNF), facilitate recovery from neuromuscular injuries. We investigated if BDNF is a key factor in MSC secretions that repairs the neuromuscular continence mechanism and improves continence in a dual injury SUI rat model.

Study design, materials and methods

Bone marrow was harvested from Sprague-Dawley rats by flushing the femur and tibia with saline. The collected cells were sorted using intracellular adhesion molecule 1 (ICAM-1) to select for MSCs and were cultured in Dulbecco's Modified Eagle Medium (DMEM) media (containing 10% fetal bovine serum (FBS), and antibiotic). BDNF expression in MSCs was knocked down (KD) by transfecting anti-BDNF shRNA via a lentivirus. A scrambled sequence was transduced as a control. Antibiotic- and serum-free media that had been cultured with the MSCs for 24 hours and contained secretions from the MSCs was collected, spun and filtered to concentrate it to 50X, producing unmanipulated control concentrated conditioned media (CCM), BDNF KD CCM, and scrambled CCM. Concentrated control media (CM) was produced in the same manner but without being conditioned by cells. All CCM and CM were stored at -80°C until used. Total protein concentration in CCM was assessed by Bradford protein assay using BSA as a standard. The concentration of BDNF in CCM was analysed by ELISA.

For creation of SUI, rats were anesthetized with 2% isoflurane and underwent pudendal nerve crush (PNC) followed by vaginal distension (VD) as described previously [1]. In brief, the pudendal nerve was isolated bilaterally and crushed with a Castroviejo needle holder twice for 30 s. The vagina was then accommodated with increasing sizes of bouge a boule urethral dilators (24 – 32Fr). A modified 10F Foley catheter was inserted into the vagina, and the balloon was inflated with 3 ml water for 4 h. Sham injury was created by making an incision in the dorsal skin, accommodating the vagina with the urethral dilators, and inserting a Foley catheter for 4 h without inflation. One hour and 1 week after the end of the sham injury (SI), rats received a 300 µl intraperitoneal injection of CM (n=17). One hour and 1 week after PNC+VD, rats received a 300 µl intraperitoneal injection of either CM (ST; n=16), BDNF KD CCM (n=12), scrambled CCM (n=11) or control CCM (n=10).

All rats underwent leak point pressure (LPP) and pudendal nerve sensory branch potential (PNSBP) recordings 3 weeks after injury, as we have described previously [1]. LPP, defined as peak bladder pressure at leakage minus baseline pressure, was used to assess urethral function. PNSBP was analysed in terms of the difference between firing rate and amplitude at peak activity during clitoral brushing and baseline activity, respectively. The urethra and pudendal nerve were harvested for anatomical assessment. ANOVA with a Student-Newman-Keuls posthoc test was used to compare quantitative outcomes with P<0.05 indicating a significant difference. Data is presented as mean ± standard error of the mean.

Results

BDNF was significantly reduced in BDNF KD CCM (48.2 ± 3.1 pg/300µl) compared to control CCM (129.0 ± 13.0 pg/300µl) and scrambled CCM (94.6 ± 8.0 pg/300µl). BDNF content in scrambled CCM was also significantly lower than that in control unmanipulated CCM. LPP was significantly decreased 3 wk after PNC+VD treated with CM (28.3 ± 2.1 cm H₂O) or BDNF KD CCM (31.1 ± 2.5 cm H₂O) compared with SI rats (40.9 ± 3.3 cm H₂O), but not with scrambled (36.0 ± 3.1 cm H₂O) or control CCM (51.1 ± 3.1 cm H₂O). Control CCM-treated rats had significantly higher LPP than the other injury groups, and the SI rats.

The amplitude of PNSBP was significantly decreased in all injury groups (SI: 0.97 ± 0.12 µV; PNC+VD with CM: 0.26 ± 0.06 µV; PNC+ VD with BDNF KD CCM: 0.61 ± 0.11 µV; PNC+ VD with scrambled CCM: 0.46 ± 0.10 µV; PNC+ VD with control CCM: 0.59 ± 0.09 µV), but the firing rate was significantly decreased only in the PNC+VD CM-treated group (399 ± 100 Hz) compared with the SI group (977 ± 93 Hz). Immunofluorescence showed healthier neuromuscular junctions in the EUS after treatment with BDNF KD CCM, scrambled CCM or normal CCM than in PNC+VD rats treated with CM. The density and diameter of pudendal nerve neurofilaments with BDNF KD CCM, scrambled CCM and normal CCM were greater than those in PNC+VD rats treated with CM.

Interpretation of results

Control CCM-treated rats performed better than SI rats in urethral function as measured by LPP testing 3 wk after PNC+VD, suggesting that control CCM can restore continence effectively, consistent with our previous study [1]. PNC+VD rats treated with scrambled CCM showed no difference in LPP from SI rats, although BDNF was decreased compared to SI rats. However, control CCM-treated rats had significantly higher LPP than the other injury groups, including PNC+VD rats treated with scrambled CCM, suggesting that lentiviral transfection even with scrambled shRNA may alter other secretions important for promotion of recovery.

In contrast, pudendal nerve recovery was facilitated by all 3 formulations of CCM tested, as indicated by improvements in PNSBP firing rate in all CCM treated groups compared to the CM treated group. However, histology findings showed better recovery in rats with BDNF KD CCM, scrambled CCM or normal CCM than ST rats, suggesting low amounts of BDNF may still be able to promote recovery of neuromuscular function but may take longer to recover to continence.

Concluding message

BDNF is an important, but not the only recovery factor in CCM for this dual muscle-nerve injury SUI animal model. Other proteins in CCM also play an important role in injury repair and recovery from SUI. Unmanipulated CCM may provide a regenerative therapy to reduce postpartum incontinence and possibly prevent later development of SUI.

References

1. Deng K, Lin DL, Hanzlicek B et al. Mesenchymal stem cells and their secretome partially restore nerve and urethral function in a dual muscle and nerve injury stress urinary incontinence model. Am J Physiol Renal Physiol. 2015 Jan 15;308(2): F92-F100.

Disclosures

Funding: The authors have no conflicts of interest. This study was supported by VA RR&D Merit Review grant I01 RX000228, the Cleveland Clinic, and Tongji Hospital. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** the Louis Stokes Cleveland VA Medical Center Institutional Animal Care and Use Committee (IACUC)