

## WNT7A IMPROVES RECOVERY IN AN INDUCED RAT MODEL OF STRESS URINARY INCONTINENCE

### Hypothesis / aims of study

Stress urinary incontinence (SUI) affects approximately 35% of women over the age of 40, reducing their quality of life [1]. SUI is strongly correlated with vaginal delivery of children and is thought to result in part from maternal neuromuscular injuries during vaginal delivery that do not fully recover [2]. While sling surgery is the gold standard treatment, up to 1/3 of patients require multiple surgeries. A Wnt7a engineered analog protein has been shown to induce muscle hypertrophy and renewal of the stem cell population [3]. The goal of this project was to assess if intraurethral injection of Wnt7A would reduce SUI in a rat model by preventing atrophy of the external urethral sphincter (EUS).

### Study design, materials and methods

Forty-three age-matched Sprague-Dawley rats underwent either sham injury or simulated childbirth injury, consisting of vaginal distension (VD) and pudendal nerve crush (PNC), which induces symptoms of SUI. The rats were divided into 4 groups: sham injury/ sham treatment (n = 11), SUI/low-dose (2.5 µg) treatment (n = 10), SUI/high-dose (10 µg) treatment (n = 10), SUI/sham treatment (n = 12). Immediately post-injury, all rats received 10µL of their respective treatment or sham treatment via direct periurethral injection. The animals underwent functional outcome testing, consisting of leak point pressure testing (LPP) and EUS electromyography (EMG) 25 days after surgery, after which the urethra and vagina were resected *en bloc*. Pressure measurements were analyzed using Astroview X, and one second segments of EUS EMG recordings at baseline and at peak were analyzed to calculate mean firing rate and amplitude of EMG activity. All groups were compared using a one way ANOVA followed by a Holm-Sidak post-hoc test. Data is presented as mean ± standard error of the mean (SEM) of each group. P<0.05 indicates a statistically significant difference between groups.

### Results

Injured rats with sham treatment had significantly decreased peak bladder pressure at leakage during LPP testing ( $38.5 \pm 3.5$  cm H<sub>2</sub>O; Figure A) compared to sham-injured, sham treated rats ( $55.4 \pm 3.8$  cm H<sub>2</sub>O; p=0.006), indicative of SUI. Peak bladder pressure at leakage in SUI rats with low-dose treatment ( $57.2 \pm 5.9$ ) was significantly increased compared to that of SUI rats with sham treatment. In contrast, there was not a significant difference in bladder pressure at leakage in SUI rats with the high dose treatment ( $43.5 \pm 3.2$  cm H<sub>2</sub>O) and SUI rats with sham treatment or between the two treatment groups.

Both animals with SUI that received high-dose treatment ( $167.4 \pm 6.5$  Hz) and sham injured, sham treated animals ( $181.8 \pm 6.1$  Hz) had an EUS EMG firing rate significantly greater than that of sham treated rats with SUI ( $126.5 \pm 13.2$  Hz; p < 0.001). SUI rats with low-dose treatment ( $136.6 \pm 11.7$  Hz) had a significantly lower EUS EMG firing rate compared to sham injured sham treated rats. There was no statistically significant difference in EUS EMG amplitude between any of the groups.

### Interpretation of results

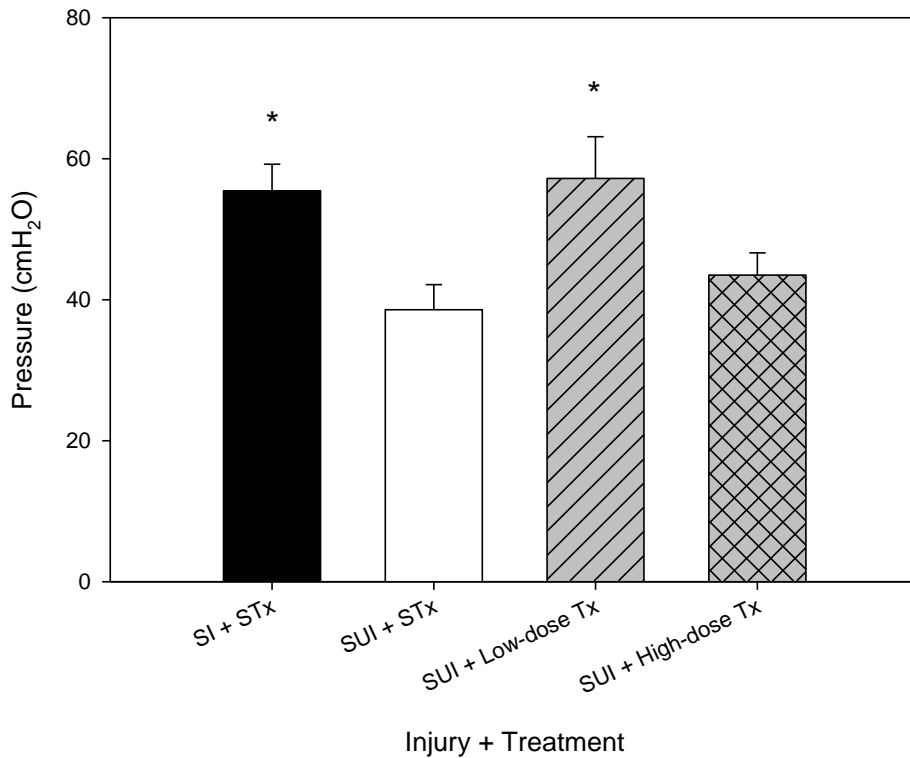
The LPP results are not mirrored in EUS EMG firing rate results, suggesting that different dosages of Wnt7A may affect the mechanism of action of Wnt7A. The low-dose treatment results demonstrate an improvement in bladder pressure at leakage compared to the high-dose treatment group, suggesting greater muscle recovery. However, the high-dose treatment results demonstrate improved neuromuscular activity compared to the low dose treatment group, as reflected in EUS EMG firing rate. Previous studies have shown that Wnt7a is a signaling protein for multiple pathways including myogenic, neuronal, and proliferative mechanisms. Variable doses of Wnt7A may target different pathways and may therefore have variable effects.

### Concluding message

SUI is a multifactorial injury with contributions from extracellular matrix, musculature, and innervation of smooth muscle and striated muscle. Single administration of Wnt7a improves the continence function of the EUS in a rat model of simulated childbirth injury; however, the mechanism of action is unclear. Further investigation is required to determine the mechanism of action. However, our findings are promising that Wnt7A may prove to be a comprehensive therapeutic and regenerative agent at a carefully selected dose.

A

### Bladder Pressure at Leakage



**Figure A:** Bladder Pressure after stress urinary incontinence injury (SUI) or sham injury (SI) and either sham treatment (STx), low-dose treatment (Low-dose Tx), or high-dose treatment (High-dose Tx). Each bar represents the mean  $\pm$  standard error of the mean of 10-12 animals. \* indicates statistically significant compared to SUI + STx.

#### References

1. Muscatello DJ, Rissel C, Szonyi, G. Urinary symptoms and incontinence in an urban community: prevalence and associated factors in older men and women. *Intern Med J.* 2001 Apr;31(3):151-160.
2. Damaser MS, Whitbeck C, Chichester P et al. Effect of vaginal distension on blood flow and hypoxia of urogenital organs of the female rat. *J Appl Physiol.* 2005 May;98(5):1884-90.
3. Le Grand F, Jones AE, Seale V et al. Wnt7a activates the planar cell polarity pathway to drive the symmetric expansion of satellite stem cells. *Cell Stem Cell.* 2009 Jun;4(6):535-47.

#### Disclosures

**Funding:** Fate Therapeutics, Inc and Cleveland Clinic Foundation **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Institutional Animal Care and Use Committee (IACUC) of the Cleveland Clinic.