

## INCREASED ACTIVITY OF $\alpha_1$ -ADRENOCEPTORS AFTER HUMAN MUSCLE-DERIVED STEM CELLS INJECTION INTO THE DENERVATED RAT URETHRA

### Hypothesis / aims of study

Autologous adult stem cell therapy for the potential regenerative repair of the deficient sphincter for the treatment of stress urinary incontinence has recently been at the forefront of incontinence research. However, the neurophysiologic mechanism of the regenerative repair remains to be investigated. The aim of this study is to elucidate the effects of pure human muscle-derived stem cells (MDSCs) injected into the denervated middle urethra of nude rats on the leak point pressure (LPP) and urethral muscle contractility.

### Study design, materials and methods

Human MDSCs obtained from biopsies of the lateral thigh in women were provided by a central approved stem cell facility. Athymic female nude rats were divided into 3 groups: (1) transected bilateral pudendal nerves near the internal iliac vessels followed by injection of 20 $\mu$ l human MDSCs suspended saline (1x10<sup>6</sup> cells/20 $\mu$ l) into the middle urethra (TM), (2) transected bilateral pudendal nerves followed by injection of 20 $\mu$ l saline into the middle urethra (TS), and (3) only exposed bilateral pudendal nerves followed by injected 20 $\mu$ l saline into the middle urethra (SS). LPP was measured 6 weeks after the injection using vertical pressure clamp methods in 2.5 cmH<sub>2</sub>O steps under urethane anesthesia (1.2 g/kg, s.c.) [Ref. 1]. In another series of experiments, concentration-dependent contractions induced by carbachol (10<sup>-7</sup> to 10<sup>-3</sup> M), a muscarinic receptor agonist, and phenylephrine (10<sup>-7</sup> to 10<sup>-3</sup> M), an  $\alpha_1$ -adrenoceptor agonist, were also examined in transverse muscle strips of the middle urethra. Contractile responses were expressed as a percent of the response to 80mM KCl, and contractile forces were adjusted with the formula, weight / (length x 1.05), where 1.05 is the assumed density of muscles.

### Results

The mean LPP of the TS group was significantly lower compared with the SS or TM group whereas the mean LPP was not significantly different in SS and TM groups (TS: 21.9  $\pm$  2.5 cmH<sub>2</sub>O < SS: 34.0  $\pm$  2.4 cmH<sub>2</sub>O  $\cong$  TM: 31.0  $\pm$  2.7cmH<sub>2</sub>O) (Fig.1). In urethral muscle strip studies, the EC<sub>50</sub> of the contractile responses to carbachol in TS and TM urethras was significantly lower compared with SS urethras whereas the differences in the EC<sub>50</sub> of carbachol responses were not significant in TS and TM urethras (TS: 2.0  $\pm$  1.3 x10<sup>-6</sup>M  $\cong$  TM: 1.7  $\pm$  1.4 x10<sup>-6</sup>M < SS: 7.6  $\pm$  1.3 x10<sup>-6</sup>M). The EC<sub>50</sub> of the contractile responses to phenylephrine in TS and TM urethras was significantly lower compared with SS urethras, and the EC<sub>50</sub> of phenylephrine responses in TM urethras was significantly lower than that of TS urethras (TM: 4.9  $\pm$  1.8 x10<sup>-6</sup>M < TS: 2.3  $\pm$  1.2 x10<sup>-5</sup>M < SS: 4.7  $\pm$  1.3 x10<sup>-5</sup>M) (Fig.2). The Emax of phenylephrine-induced contractile forces of TM urethras was significantly higher compared with SS whereas the differences in the Emax of SS and TS urethras were not significant (TM: 0.11  $\pm$  0.02 g/mm<sup>2</sup> > SS: 0.06  $\pm$  0.02 g/mm<sup>2</sup>  $\cong$  TS: 0.09  $\pm$  0.02g/mm<sup>2</sup>). In addition, the Emax of carbachol-induced contractile forces of SS, TS or TM urethras were not significantly different (SS: 0.16  $\pm$  0.03 g/mm<sup>2</sup>  $\cong$  TS: 0.20  $\pm$  0.05 g/mm<sup>2</sup>  $\cong$  TM: 0.16  $\pm$  0.02 g/mm<sup>2</sup>) (Fig.3).

### Interpretation of results

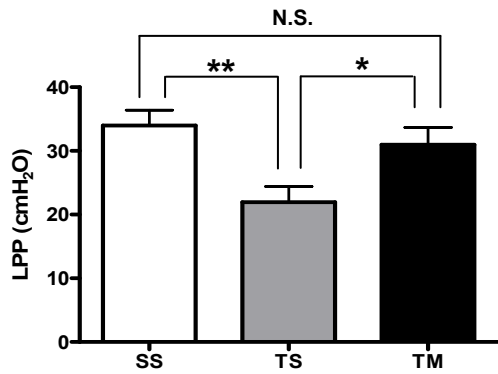
In this study, the mean LPP in pudendal nerve-injured rats injected with human MDSCs (TM group) was increased to the same level as in sham rats (SS group) 6 weeks after the injection. In addition, the binding affinity (EC<sub>50</sub>) and efficacy (Emax) of  $\alpha_1$ -adrenoceptors in nerve-injured urethral strips with human MDSCs injection (TM group) were also increased compared with sham rats (TS and SS groups). These results suggest that human MDSCs can increase sensitivity and the number of  $\alpha_1$ -adrenoceptors, but not those of muscarinic receptors, in the denervated urethral sphincter of the middle urethra. This mechanism may contribute to regeneration and functional recovery of the denervated urethra, leading to enhanced urethral closure evidenced by high LPP following MDSCs injection.

### Concluding message

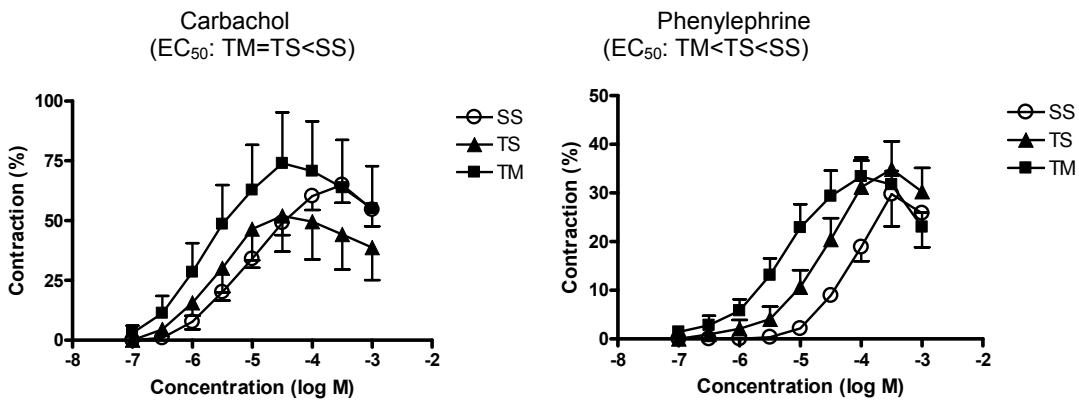
MDSCs injection into the middle urethra that can increase contractility of the deficient urethral sphincter mediated by enhanced activity of  $\alpha_1$ -adrenoceptors in the middle urethra will be useful for the treatment of stress urinary incontinence.

### References

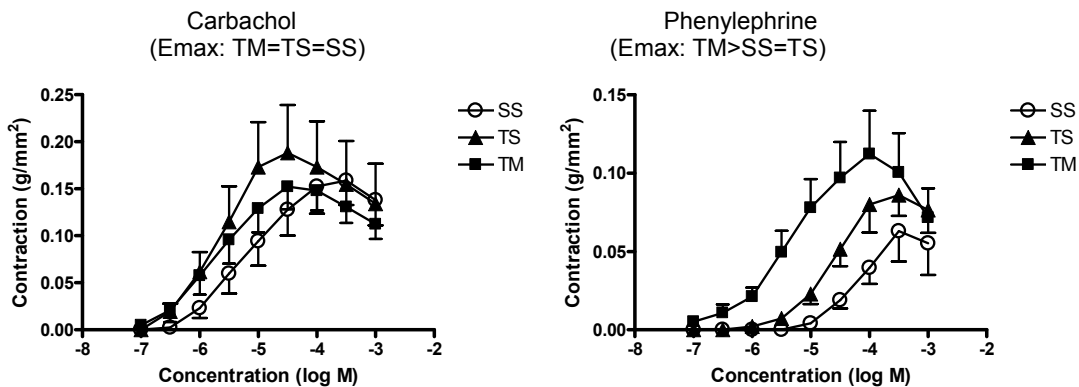
1. Int Urogynecol J 2003; 14: 31-7.



**Fig. 1.** Comparative effects of human MDSCs injection on the LPPs of SS, TS and TM. \*P<0.05 and \*\*p<0.01.



**Fig. 2.** Comparison of concentration-response curves in response to carbachol and phenylephrine in SS, TS and TM urethral strips.



**Fig. 3.** Comparison of contractile forces in response to carbachol and phenylephrine in SS, TS and TM urethral strips.

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**ANIMAL SUBJECTS:** This study followed the guidelines for care and use of laboratory animals and was approved by University of Pittsburgh Institutional Animal Care and Use Committee