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LOCAL ELECTRICAL STIMULATION WITH MESENCHYMAL STEM CELLS IMPROVES ANATOMY AND FUNCTIONAL RECOVERY LONG AFTER ANAL SPHINCTER INJURY IN A RAT MODEL.

Hypothesis / aims of study
We have previously optimized electrical stimulation (ES) parameters for up-regulation of mesenchymal stem cell (MSC) homing cytokines in the anal sphincter in a rat model. Our hypothesis is that low grade ES acts as a conditioning injury which upregulates homing cytokines that attract and retain MSC at the the area of previous injury thereby stimulating regeneration. The aim of this study was to evaluate if local ES with allogeneic MSC delivery has an effect on anal sphincter anatomy and physiology long after anal sphincter injury in a rat model.

Study design, materials and methods
16 virgin female age and weight-matched Sprague Dawley rats had a large anal sphincter excision (50% of the circumference of both the internal and external anal sphincter muscles). Three weeks after injury, animals were randomly allocated to four groups (n=4 each group) based on different treatment methods: injury alone (IA), injury + daily ES for 3 days (3ES), injury + daily ES for 3 days with MSC injection (10^6 cells) once on Day 3, 1 hour after ES (ES+1MSC); and injury + daily ES for 3 days with two injections of MSC on Day 1 and Day 3, 1 hour after ES (ES+2MSC). ES parameters were selected based on our previous study: 0.25mA current /40 Hz frequency /100μs pulse duration/60min stimulation duration. The time points of observation for anal sphincter pressures and histology were pretreatment and 4 weeks after treatment. Image-Pro Plus 7.0 was used to quantitatively assess percentage of new muscle and connective tissue regeneration in the area of injury, normalized to the uninjured area in the same animal on Masson Trichrome stained transverse sections.
Statistical significance ($p<0.05$) was determined using ANOVA on Ranks with a Tukey test for both manometry and histology results.

Results
Manometry results indicate that ES+1MSC showed significantly improvement ($p<0.05$) in the mean resting pressure (8.7±4.6 cmH_2O,) compared with IA (4.1±5.3 cmH_2O), 3ES (5±7.3 cmH_2O), and ES+2MSC (6.1±4.5 cmH_2O). No significant difference was found in either average contraction pressure or maximum contraction pressure among the treatment groups ($p>0.05$). Histology showed that ES+MSC significantly increase the anal sphincter muscle in the area of injury (105%±40%) compared with IA (71% ±17%) ($p<0.05$). There was no obvious difference when ES (85%±24%) was compared with IA (71%±17%), or ES+MSC (105%±40%) and ES+2MSC (77.4%±32%) ($p>0.05$). (Figure). There was no significant difference was found among groups (IA 100%±22%, 3ES 84% ± 36%; ES+MSC 121% ± 69%; ES+2MSC 84±46%, $p>0.05$) in the connective tissue.

Interpretation of results
Electrical stimulation acts as a conditioning injury. ES upregulates certain homing cytokines which attract MSC to the site of stimulation. In this experiment the site of stimulation was the site of previous injury. MSC were retained at the site of previous injury. When multiple days electrical stimulation (3 days) was used followed by one or 2 doses of MSC injection it was found that the effect on the anal manometry and histology were not increased with increased doses of MSC. New muscle formation was a result of ES+MSC which was not seen in the injury without any treatment or ES alone.

Concluding message
In this rat model of a chronic large anal sphincter injury, ES with a single local MSC delivery significantly improved anal sphincter function and anatomy over multiple MSC injection. Therapeutic implications include the possibility of using ES at a time remote from injury as a stimulus for MSC directed muscle regeneration. Future studies can involve a proof of concept clinical study using serial ES over multiple days followed by autologous MSC.

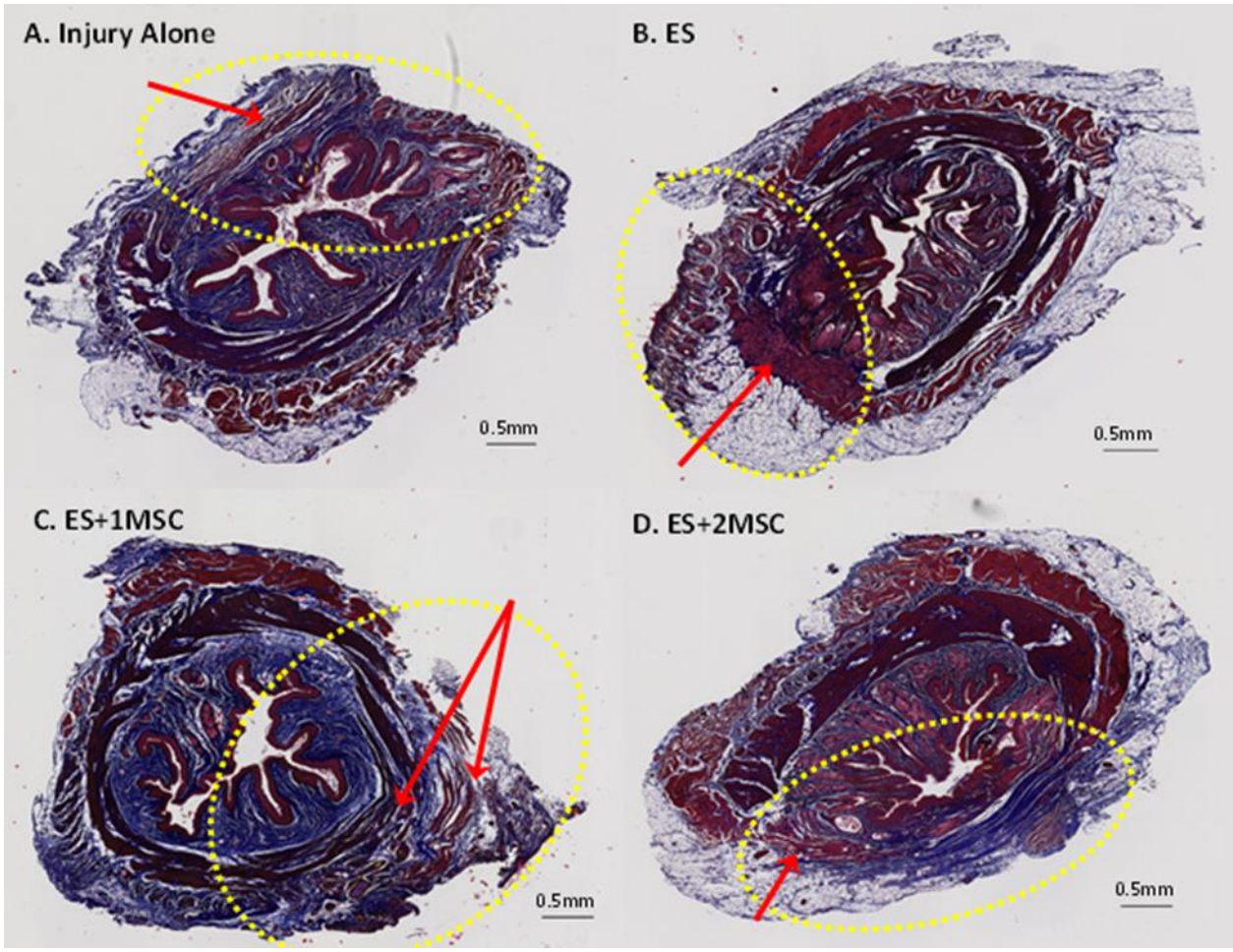

Figure legend.
Transverse sections of the anal canal stained by Masson Trichrome after a 50% anal sphincter excision followed by electrical stimulation (ES) for 3 days and treatment with two different doses of mesenchymal stem cells (MSC) or no treatment 3 weeks after injury.
A: Area of injury without treatment. B: 3-day ES treatment alone (3ES). C: 3-day ES with one time MSC treatment (ES+1MSC). D: 3-day ES with two time MSC treatment (ES+2MSC). New muscle is indicated by red arrow in the injured area (circled by yellow.

References

1. Salcedo L, Penn M, Balog B, Damaser, Zutshi M . Functional outcome after anal sphincter injury and treatment with mesenchymal stem cells. Stem Cell Translational Medicine. 3(6):760-7
2. Damaser, M.S., L. Salcedo, G. Wang, P.J. Zaszczurynski, M.A. Cruz, R.S. Butler, H.-H. Jiang, and M. Zutshi (2012) Electrical stimulation of anal sphincter or pudendal nerve improves anal sphincter pressure. Diseases of Colon & Rectum 55(12):1284-94.
3. Salcedo L, Lei L, Jiang H, Sopko N, Penn M, Damaser M, Zutshi M. Low current electrical stimulation upregulates cytokine expression in the anal sphincter. Int J Colorectal Dis .2012(2) 221-5

Disclosures

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