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Zutshi M[1], Salcedo L[1], Penn M[1], Mayorga M[1], Hull T[1], Damaser M[1]
1. *Cleveland Clinic Foundation*

MESENCHYMAL STEM CELLS IMPROVE FUNCTIONAL OUTCOME AFTER ANAL SPHINCTER INJURY IN AN ANIMAL MODEL

Hypothesis / aims of study

Fecal incontinence continues to affect a considerable number of women secondary to direct anal sphincter trauma and pudendal nerve damage after childbirth. Current conservative and surgical therapies do not offer long term cure. Mesenchymal stem cell (MSC) treatment for tissue repair and regeneration has been widely studied and initial reports showed promising results (1).
Hypothesis. In response to tissue injury stem cells are drawn to the area and contribute to tissue repair.
Aim. This study aims to evaluate MSC engraftment after direct or intravenous injection and study anal sphincter pressures after injury and treatment with MSC in our previously created animal model of injury (2).

Study design, materials and methods

Forty virgin female Sprague Dawley rats were randomly divided into direct injection through the anal sphincter (DD, n=20) and intravenous (IV, n=20) tail vein injection groups. After adequate anaesthesia, two forms of injury model were studied. Sphincterotomy (AS) was done with a precise cut in the anal sphincters. Pudendal nerve crush (PNC) was performed through the ischiorectal fossa. The pudendal nerves were isolated and crushed twice for 30s each with a needle holder. Each injection route group (DD or IV) consisted of subgroup of AS with saline treatment (n=5), AS with MSC treatment (n=5), PNC with saline treatment (n=5) and PNC with MSC treatment (n=5). All animals underwent anal pressure testing before and immediately after injury using a saline filled balloon connected to a pressure transducer and a digital amplifier. Both resting and peak pressures were recorded accordingly. Twenty four hours after injury, each subgroup was injected with either 0.2ml saline or 2 million rat MSC suspended in 0.2ml PBS (n=5) labelled with green fluorescing protein (GFP) with a passage of 18 to 25. Animals were followed up to 10 days post injury and a repeat testing post-injury (PI) for anal pressure was done. Euthanasia was carried out and anal sphincters were harvested and fixed for immunostaining with GFP antibody and nuclear stain. Anal pressures were statistically analyzed using a t-test with $p<0.05$ indicating significant difference. Results are presented as mean ± standard error of the mean of data from 5 animals.

Results

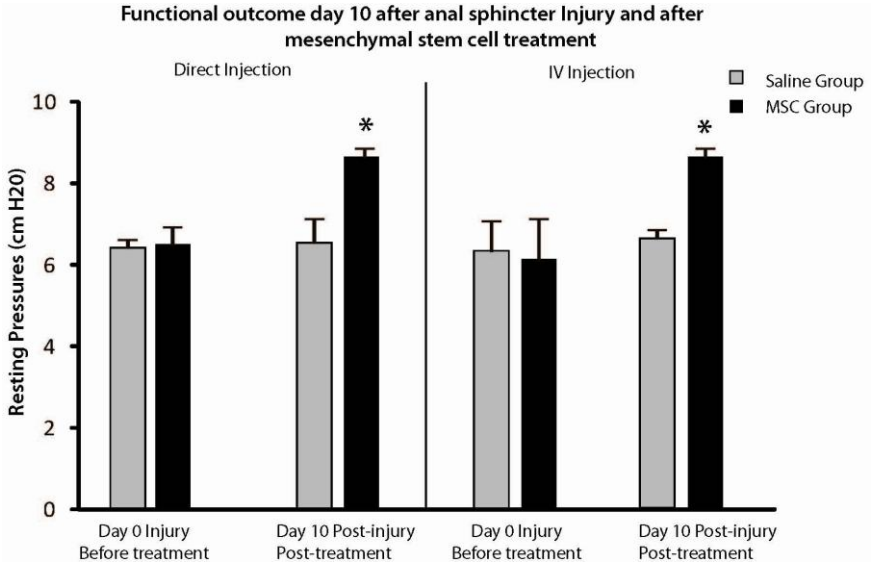
Anal pressures declined immediately after. Ten days post-injury, the MSC-treated DD(AS) group had significantly increased pressures compared with the saline-treated DD(AS) group. Resting anal pressures were increased from 6.5 ± 0.4 to 8.6 ± 0.2 (MSC treated) vs.6.4 ± 0.2 to 6.5 ± 0.6 cmH_20 (saline treated DD AS) (p=0.03) and peak pressures were increased from 7.5 ± 0.3 to12.9 ± 1.3(MSC treated) vs. 7.5 ± 0.3 to10.7 ± 0.9 cmH_20 (saline treated DD AS) (p=0.04). In the IV(AS) group, resting and peak pressures were also significantly increased compared to saline-treated animals: 6.1 ± 1.0 to 8.6 ± 0.2 (MSC treated) vs. 6.4 ± 0.7 to 6.6 ± 0.2 (saline treated IV AS) (p<0.001), 7.2 ± 0.8 to 11.9 ± 0.5 (MSC treated) vs. 7.3 ± 0.9 to9.6 ± 0.5 (saline treated IV AS) (p=0.01),respectively (Figure). The PNC group did not show significant improvement with either MSC or saline treatment. Immunostaining showed more fluorescing GFP-labelled cells surrounding the external anal sphincter in the MSC-treated animals after AS. There was no significant difference in the number of cells that engrafted after direct injection and IV infusion.

Interpretation of results

Anal pressures improved after MSC injection given post injury which indicates its potential to increase functional outcome by virtue of sphincter augmentation or increase in expression of growth factors in the anal sphincter (1). MSC homing ability might be attributed to several factors like the presence of specific receptors present on their cell surface and its ability to recognize the chemokines expressed by the anal sphincter in response to injury (3).

Concluding message

GFP labelled MSC cells home to the external anal sphincter after mechanical injury. MSC engraft in the injured anal sphincter after both direct and IV infusion. Significant improvement in anal pressures was seen in the MSC-treated group post sphincterotomy but not after nerve crush.



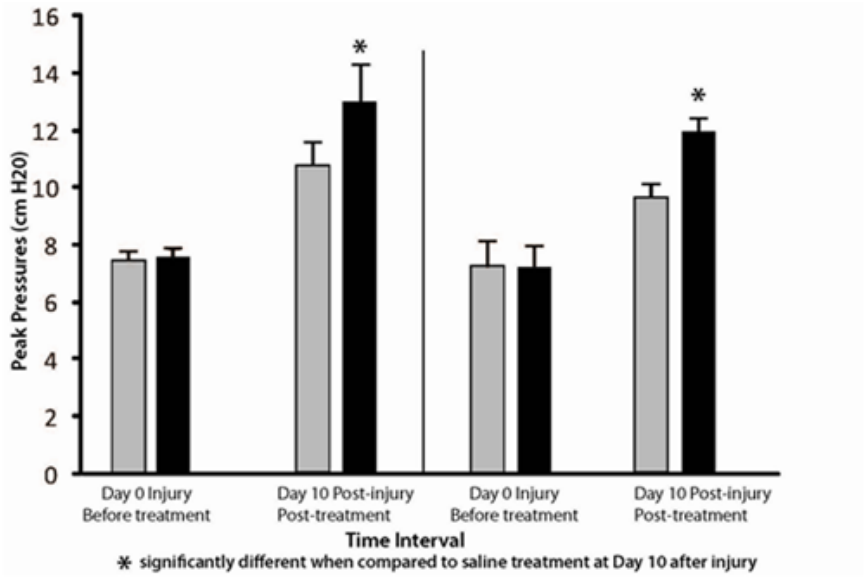


Figure. Anal sphincter pressures before and after direct anal sphincter injury and treatment with rat mesenchymal stem cells and compared with controls (saline treated) at various time points.

References

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)**