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SERIAL IV INFUSIONS OF STEM CELLS CAUSE SUSTAINED ANAL PRESSURE IMPROVEMENT AFTER ANAL SPHINCTER INJURY.

Hypothesis / aims of study

Damage to the anal sphincter during delivery of children can lead to fecal incontinence, a condition that is devastating to quality of life. Stem cells have the potential to facilitate recovery from this damage and treat or prevent fecal incontinence. Intravenous (IV) delivery of stem cells may provide a less invasive delivery route if they home to areas of injury and facilitate recovery. The hypothesis of this study was that mesenchymal stem cells (MSC) will home to the injured anal sphincter and facilitate restoration of continence. We tested the hypothesis by comparing functional and anatomic outcomes of intramuscular (IM) and IV administration of MSCs in an animal model of anal sphincter injury.

Study design, materials and methods

After approval from the Institution for animal and use committee, 45 virgin rats were divided into injury (n=35) and no injury (NI , n=10) groups. The injury group was divided into saline (PBS) or MSC treatment and a control group (n=5) which received no treatment. Each treatment group was further divided into IM (direct injection into the region of the injured anal sphincter) and serial IV infusion (n=5) group . The MSC IM and IV (n=5) and control groups were followed up for 5 weeks.

The injury was a partial anal sphincter excision (PSE) of 25% of the anal sphincter. Anal pressures were recorded prior, 10 days and 5 weeks after treatment with a balloon connected to a digital recorder. Twenty four hours after injury, the animals received $5x10^6$ labelled MSC or 0.2ml saline into the anal sphincter for IM treatment , while IV treatment group received the same dose of MSC /saline daily for 6 consecutive days via the tail vein. Anal sphincters were harvested and submitted for Masson's staining.

Statistical analysis

Anal pressures were measured as resting and peak pressures based on our animal model[1].

The anal pressures were analyzed with one way ANOVA and Tukey Kramer posthoc all pairwise comparisons were performed. P values were set at <0.05 to indicate significant difference. Values are presented as means ± standard errors.

Results

IM treatment group:

Ten days after IM treatment, significant increase in resting (P<0.001) and peak pressures

(P<0.001) was seen after MSC treatment when compared with PBS after injury (9.78 ± 0.84, 13.13 ± 1.2, respectively) vs. (6.23 ± 0.48, 8.32 ± 0.64, respectively). When comparing the , the IM MSC treatment with the NI group, recovery of the anal pressures was not complete (resting (P=0.04) and peak pressures (P=0.02) with pressures.

IV treatment group

The IV infusion group showed significantly increased resting (P<0.001) and peak pressures (P<0.001) in MSC treated animals compared with PBS (11.03 ± 0.71, 16.68 ± 1.33, respectively) vs. (6.94 ± 0.28, 8.56 ± 0.34, respectively) after injury (Figure). However recovery was complete and no significant difference was seen in the pressures when compared to NI group.

Results at 5 week time period.

Five weeks after IM treatment significantly increased peak pressures (P=<0.001) after MSC treatment were seen compared to NI group. However, after IV treatment significantly increased resting (P=0.01) and peak pressures (P=<0.001) were seen compared to the control group. Marked decrease in fibrosis and scar tissue was seen in the MSC treated group with the least fibrosis seen in the MSC IV infusion treated group.

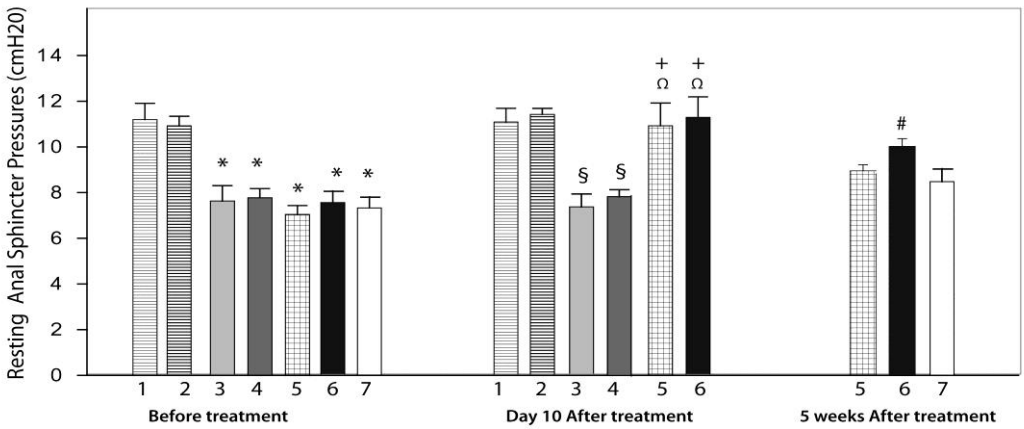

Figure legend:

Comparison of resting pressures after anal sphincter injury, before and after treatment with MSC or saline given IM or via serial IV infusions and a no treatment group.

1-Control (No injury) + IM MSC ; 2-Control (No injury) + IV MSC; 3-PSE (injury) + IM PBS; 4-PSE (injury) + IV PBS; 5-PSE (injury) + IM MSC; 6-PSE (injury) + IV MSC; 7-PSE (injury) + No Tx;

PSE= Partial Anal Sphincter Excision TX= TREATMENT
*-significantly decreased before treatment when compared to Control
§- significantly decreased at Day 10 after treatment when compared to Control
Ω - significantly increased at pre-treatment when compared to PSE (MSC)
+- significantly increased at Day 10 after-treatment when compared to PSE (saline)
#-significantly increased at 5 wks when compared to PSE (no injury,no treatment)

Interpretation of results

Anal sphincter pressures declined after PSE with PBS treatment. Ten days after treatment with MSC via either IM or IV routes, anal pressures increased significantly with a greater increase after IV infusion. Five weeks after PSE and MSC IV treatment, anal pressures remained significantly high compared to a PSE group that received no treatment. Histology demonstrated that the process of healing is by fibrosis, which is markedly reduced in the IV infusion group that received MSC.

Concluding message

Although direct injection (IM) of MSC into the anal sphincter muscle after injury facilitates an increase in anal pressures, it is not sustained 5 weeks later to the same extent as after treatment with IV infusion. Healing is by fibrosis in the PBS treated animals while the MSC treated groups showed less scarring, particularly after IV infusion of MSC. IV infusion appeared to confer more benefit than a single IM injection of MSC in the anal sphincter muscle. This may be due to the greater number of cells utilized and greater duration of treatment in the IV infused group. This study suggests the potential effectiveness of a clinical study utilizing multiple IV infusions of MSC after anal sphincter injury.

References

1. Zutshi, M,Salcedo, L, .Zaszczurynski, .Hull, T,Butler, R,Damaser M.Effects of sphincterotomy and pudendal nerve transection on the anal sphincter in a rat Dis Colon Rectum, 2009. 52(7): p. 1321-9.

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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	**Institution of Animal Care and Use Committee (IACUC)**