

SDF-1 PLASMID REGENERATES BOTH SMOOTH AND SKELETAL MUSCLE AFTER ANAL SPHINCTER INJURY IN THE LONG TERM

Hypothesis / aims of study

Regenerating muscle at a time remote from injury requires re-expression of cytokines to attract stem cells to start and sustain the process of repair. We have demonstrated that a non-viral plasmid expressing stromal cell-derived factor 1 (SDF-1) increased muscle regeneration 4 weeks after treatment following a large chronic anal injury. Our aim was to evaluate the sustainability of this muscle regeneration and evaluate the muscle morphology and molecular mechanisms that lead to it.

Study design, materials and methods

56 female age/weight-matched Sprague-Dawley rats underwent an excision of 50% of the circumference of the anal sphincter complex. 3 weeks after injury, rats were randomly allocated to 1 of 4 groups (n=8) based on treatment: injury with no intervention (IA); 100 μ g plasmid encoding SDF-1 injected locally (P); both plasmid and 8 \times 10⁵ mesenchymal stem cells (MSC) injected locally (P+MSC) and plasmid injected locally with injection of a gelatin scaffold mixed with MSC (P+S&MSC). Rats underwent anal manometry pre-excision and pre and post-treatment. ImagePro Plus 7.0 was used for histological quantification by comparing muscle regenerated and fibrosis at the site of defect with intact muscle in the same section at 8 weeks. Immunohistochemistry used Desmin antibody for muscle identification. Protein expression of CXCR4 (receptor of SDF-1) and skeletal muscle proliferation/differentiation marker Myf5 was done by Western Blot at day 7 post treatment. One way ANOVA followed by Tukey test was used for data analysis (mean \pm SD), p<0.05 was regarded as significant.

Results

Pre-treatment anal pressures were not significantly different among groups. 8 weeks post-treatment all 3 groups receiving the plasmid had significantly higher pressures (cmH₂O) (P:10.9 \pm 2.11, p=0.006, P+MSC: 13.1 \pm 7.07, p=0.04, P+S&MSC:10.6 \pm 3.70, p <0.001) than the IA group (3.4 \pm 0.96). Qualitative assessment of histology revealed that the plasmid alone group showed more organized muscle architecture in the entire circumference of the defect. Muscle quantification showed that compared to IA (0.9 \pm 0.06) only the plasmid alone group had significantly more muscle in the area of the defect (P: 1.0 \pm 0.09, p=0.03). On comparing the connective tissue the plasmid alone group (1.1 \pm 0.07) had significantly less fibrosis than the IA (1.4 \pm 0.24, p=0.02) and P+S&MSC groups (1.4 \pm 0.21, p=0.03). Immunohistochemistry showed that there was no change in the ratio of new smooth to new skeletal muscle among groups with treatments (p0.05) (Figure). There were no significant differences in protein levels of CXCR4 or Myf5 between groups 7 days after treatment.

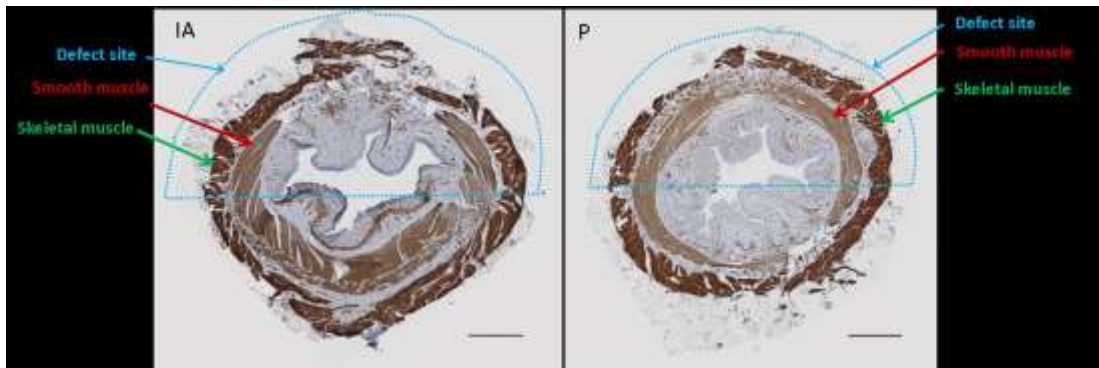


Figure. Representative pictures of transverse sections of rat anal canal stained by Desmin antibody. These sections are 3 weeks post-excision of 50% of the circumference of the anal sphincter and treated with injury without treatment (IA)(right) and SDF-1 plasmid local injection at the site of the defect (P) (left). There is regeneration of both smooth and skeletal muscle which is organized in the plasmid treated group. In the area of the defect (circled by blue oval), smooth muscle is indicated by red arrow and skeletal muscle is indicated by green arrow. Scale bar= 1mm.

Interpretation of results

SDF-1 plasmid increased anal sphincter pressures 8 weeks post-treatment to near pre-excision levels. SDF-1 plasmid alone also regenerated both smooth and skeletal muscle and decreased fibrosis at the site of defect. The injury alone group decreased in the pressures and had disorganized muscle regenerated. The other two groups using the SDF-1 plasmid also had a positive effect on both anal pressures and regeneration of smooth and skeletal muscle. The cytokines CXCR4 and Myf5 were not elevated indicating that the SDF-1 plasmid may not be effecting the regeneration through expression of these cytokines.

Concluding message

In a model of chronic injury, SDF-1 plasmid alone regenerated both smooth and skeletal muscle and decreased fibrosis at the site of defect. This resulted in elevating sphincter pressures to normal levels. This effect could not be attributed to a change in the expression of cytokines CXCR4 and Myf5

References

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Disclosures

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