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INTRAVENOUSLY INJECTED MESENCHYMAL STEM CELLS HOME TO PEVIC ORGANS AFTER SIMULATED CHILDBIRTH INJURY

Hypothesis / aims of study

During the second stage of vaginal delivery, pressure of the fetal head on the pelvic floor causes direct trauma to the pelvic muscles, the urethral and anal sphincters, and the nerves that innervate them. These injuries can lead to the development of pelvic floor disorders such as pelvic organ prolapse (POP), urinary incontinence and fecal incontinence [1].There is no therapy at the present time that facilitates repair after childbirth injuries and prevents the occurrence of pelvic floor disorders. Stem cell therapy has increasingly been focused on for the treatment and possible prevention of pelvic floor disorders. It has been demonstrated that a stem cell homing cytokine, chemokine (C-C motif) ligand 7 (CCL7), alternatively named MCP-3, and one of its receptors are upregulated in the urethra and vagina of rats after childbirth injury [2], suggesting that MSCs delivered systemically after delivery will home to these organs and facilitate repair. The aim of this study was to investigate if intravenous (IV) allogenic bone marrow-derived mesenchymal stem cells (MSCs) home to the pelvic organs after simulated childbirth injury.

Study design, materials and methods

Age-matched virgin female Sprague-Dawley rats underwent either a simulated childbirth injury by vaginal distension (VD; n=11) or sham VD (n=11). For VD, each rat was anesthetized, a modified 10Fr Foley catheter was inserted into the vagina and the balloon was inflated to 3 ml for 4 hours. Sham VD consisted of catheter insertion for 4 hours without balloon inflation. Both groups received 2 million GFP-labelled MSCs injected via the lateral tail vein 1 hour after injury. Four or 10 days later, rats in each group were anesthetized & imaged *in vivo* for visualization of GFP-positive cells using a supercooled charge-coupled camera in a light tight box after which the urinary bladder, urethra, vagina, rectum and levator ani muscles were harvested from each animal and imaged similarly *ex vivo*. Total fluorescent flux (photons/second/cm^2/steridian) in a region of interest selected around each organ from *ex vivo* imaging was calculated. Values from VD animals were normalized to that of a paired sham VD animal which was imaged simultaneously. Quantitative values are presented as mean ± standard error of the mean. Statistical comparisons were made using a Student's t-test with $p<0.05$ indicating a significant difference between groups. *In vivo* imaging data was analyzed qualitatively.

Results

In vivo imaging demonstrated GFP-positive MSCs in the pelvic region of rats after VD (Figure 1). Total flux of fluorescence in the urethra, vagina, rectum, and levator ani imaged *ex* vivo 4 days after VD was significantly greater than after sham VD (Figure 2). Only total flux of fluorescence in the urethra *ex vivo* 10 days after VD was significantly greater than after sham VD. There was no significant difference in total flux of the urinary bladder between VD and sham VD either 4 or 10 days after injury. Additionally, there was a significant decrease in total flux from 4 to 10 days after VD in the rectum and urethra.

Interpretation of results

In vivo imaging demonstrates regions positive for GFP fluorescence but cannot be used to identify individual organs. *Ex vivo* imaging demonstrated that MSCs home to the urethra, vagina, rectum and levator ani muscle after simulated childbirth injury. Although the total flux of fluorescence was significantly decreased 10 days after injection, possibly indicating a lower cell density, the MSCs were retained to a significant degree in the urethra only.

Concluding message

Intravenously injected MSCs home to most pelvic organs after simulated childbirth injury. Although they are not retained long in most organs, other studies have shown a therapeutic effect in a short time [3]. IV delivery is a potentially effective and noninvasive route for the application of stem cell therapy.

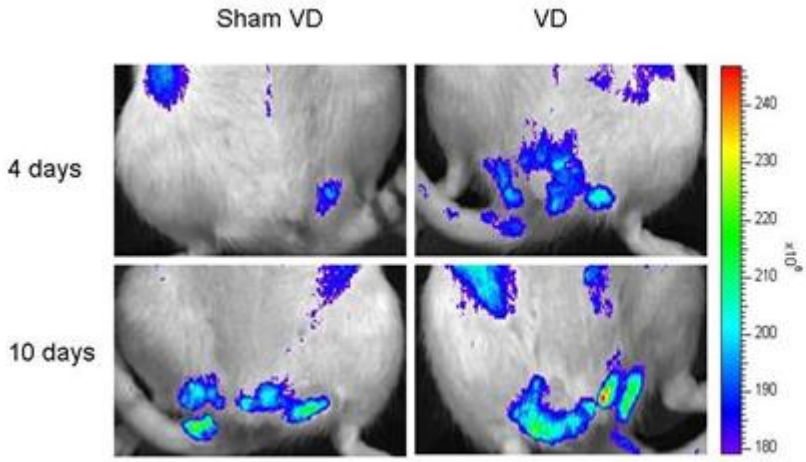

Figure 1. *In vivo* imaging of GFP-positive MSCs 4 and 10 days after either vaginal distension (VD) or sham VD and MSC injection. The colored bar indicates the level of total fluorescent flux in a region.

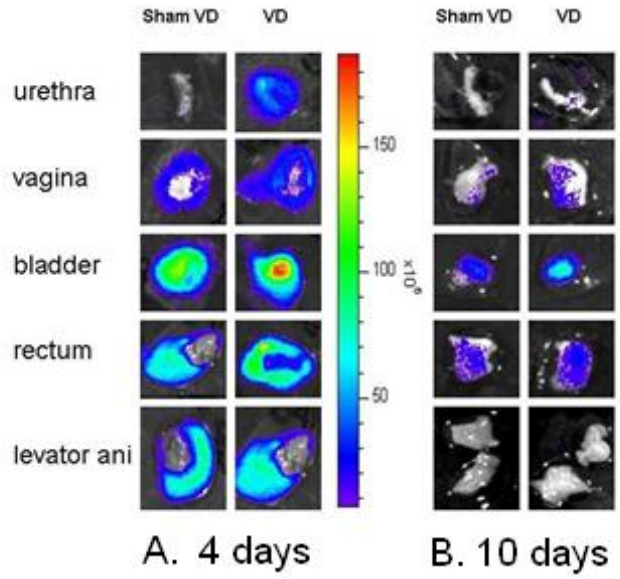

Figure 2. *Ex vivo* imaging of GFP-positive MSCs in each organ **A.** Four days after either vaginal distension (VD) or sham VD & MSC injection and **B.** Ten days after VD or sham VD and MSC injection. The colored bar indicates the level of total fluorescent flux in a region.

References

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Is this a clinical trial?	**No**
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	**The Cleveland Clinic Institutional Animal Care and Use Committee**