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STEM CELL HOMING CYTOKINE UPREGULATION IN LYSYL OXIDASE LIKE 1 (LOXL1) KNOCKOUT MICE AFTER VAGINAL DISTENSION

Hypothesis / aims of study

Vaginal distension (VD) has been used in rodents to simulate human maternal childbirth injuries. Adult mesenchymal stem cells (MSCs) home to the urethra in female rats after VD due to upregulation of stem cell homing cytokines in pelvic tissues and can accelerate recovery [1]. Unlike the rat animal model, Lysyl Oxidase Like-1 Knockout (LOXL1 KO) mice develop female pelvic floor disorders (FDFD) after pregnancy and vaginal delivery, including pelvic organ prolapse [2,3]. The objective of this study was to determine if cytokines and cytokine receptors involved in stem cell homing and tissue repair are differentially expressed by pelvic floor tissues after VD in LOXL1 KO mice.

Study design, materials and methods

Age-matched, nulliparous, virginal LOXL1 KO mice were assigned to either the VD (n=3) or control (n=4) groups. All were anesthetized for 6 hours via intraperitoneal injection of a mixture of ketamine/xylazine. In the VD group, the vagina was serially dilated using urethral dilators, a pediatric foley catheter was inserted, and the balloon was distended with 0.3 mL for 6 hours. In the control group, no vaginal distension was performed. After 6 hours, all mice were sacrificed and underwent pelvic dissection. The urethra, anterior vaginal wall, and bladder base were harvested, immediately snap frozen, and stored at -80 °C. Quantitative real-time RT-PCR was performed on RNA extracted from the urogenital organs. mRNA expression of the stem cell homing cytokines and receptors was normalized to GAPDH: monocyte chemotactic protein-3 expression (MCP-3) and stromal derived factor-1 (SDF-1) and a receptor for MCP-3, chemokine receptor-1 (CCR-1). mRNA expression in control and VD groups were statistically compared using a Student's t-test with $p<0.05$ indicating a significant difference. mRNA expression of animals in the VD group are presented relative to mean expression of control animals in the same tissue.

Results

MCP-3 expression was increased 15 fold in the vagina (p=0.03), 5.3 fold in the urethra (p=0.19), and 1.8 fold in the bladder (p=0.27) after VD compared with controls (Figure 1A). SDF-1 expression was increased 1.8 fold in the vagina (p=0.30), but was unchanged in the urethra (p=0.46) and bladder (p=0.64) (Figure 1B). CCR-1 expression was increased 4 fold in the vagina (p=0.02) and 1.8 fold in the urethra (p=0.09), and unchanged in the bladder (p=0.79) (Figure 1C).

Interpretation of results

Both MCP-3 and its receptor CCR-1 are significantly over expressed in LOXL1 KO mouse vaginal tissues immediately following vaginal distension, consistent with previous work with Sprague Dawley rats [1]. As in rats, SDF-1 expression is not upregulated. In contrast to the studies in rats, vaginal expression of MCP-3 and CCR-1 is greater than urethral expression, correlating with functional differences in these animal models since the mice develop pelvic organ prolapse and the rats do not.

Concluding message

Our findings suggest that stem cell homing plays an important role in recovery from simulated childbirth injury in LOXL1 KO mice. Increased understanding of the role of MCP-3 and its receptor CCR-1 over expression in targeted stem cell migration could contribute to the development of novel treatments and/or preventive measures for stress urinary incontinence.

Figures

Figure 1A

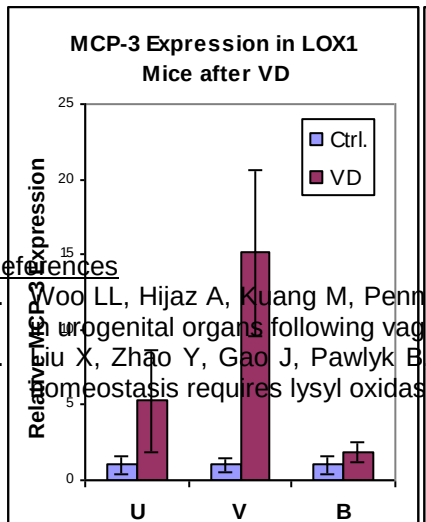


Figure 1B

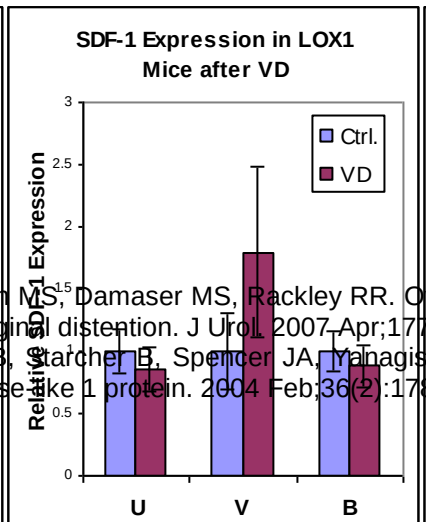
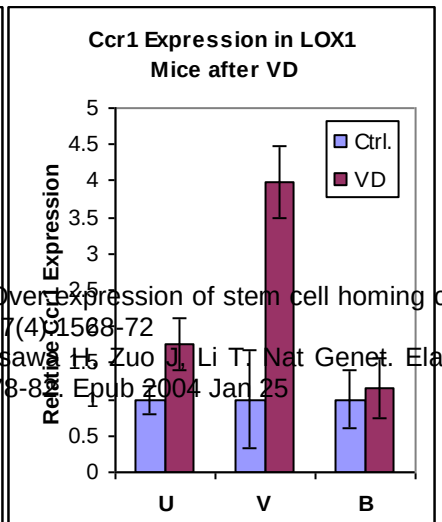


Figure 1C



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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	**Cleveland Wade Park VA IACUC**