

CHANGES IN VAGINAL DE NOVO EXTRACELLULAR MATRIX ASSEMBLY IN THE LYSYL OXIDASE LIKE – 1 (LOXL1) KNOCKOUT (KO) MOUSE MODEL OF PELVIC FLOOR DISORDERS

Hypothesis / aims of study

Deficient remodelling of the extracellular matrix (ECM) of pelvic structures during pregnancy and in the post-partum period could be related to female pelvic floor deficiency, resulting in pelvic floor disorders (PFD), including stress urinary incontinence and pelvic organ prolapse (POP). Elastin and collagen critically determine soft tissue mechanics and intracellular cell-signalling, and their turnover is determined by several biological factors, including matrix metalloproteinases (MMP), and tissue inhibitors of MMP (TIMP) [1]. In the lysyl oxidase like-1 (*Loxl1*) knockout (KO) mouse model of PFD, the condition develops after vaginal delivery and appears to correlate with disorganized elastin clusters, and abnormal ECM repair. To establish a pathophysiologic basis for PFD development, we compared *de novo* ECM assembly between wild type (WT) mice and *Loxl1* KO mice with and without POP, as an indicator of PFD.

Study design, materials and methods

Mouse pelvic organ prolapse quantification (MOPQ) was used to assess POP [2]. Mice with grade 0 or 1 were classified as non-prolapsed, and those with grade 2 or 3 were classified as prolapsed. Vaginal wall tissue was harvested from age- and parity-matched *Loxl1* KO multiparous prolapsed (MP) (n=3), and multiparous non-prolapsed (MNP) mice (n=3); and age-matched *Loxl1* KO nulliparous (N) (n=3) and WT mice (n=3). The mice were anesthetized with isoflurane, the pubic symphysis cut to expose the urethra and vagina, then bladder, urethra and rectum were carefully dissected from the vagina, which was resected at the level of the cervix on its proximal end, and close to the skin on its distal end. Primary smooth muscle cells (SMC) were isolated by elastase/collagenase digestion of these vaginal explants and assessed for expression of the SMC phenotypic markers α -actin, SM22, caldesmon, smoothelin, and tropomyosin. Passage 2 SMCs from each group (3000 cells/cm²; n = 6 replicate cultures/cell type/assay) were cultured for 21 days in DMEM/F12 medium with 10% fetal bovine serum. Fluorometric DNA assay for SMC proliferation and Fastin assay for elastic matrix content (normalized to DNA content) were done. mRNA was isolated from cell cultures, then RT-PCR was performed to assess the expression of tropoelastin (Eln), collagen 1a (Col1a), MMP2, MMP9, TIMP 3, TIMP4, lysyl oxidase (Lox), fibulin 5 (Fbln5), and fibrillin 1 (Fbn1).

Data Analysis. One-way ANOVA was used to compare DNA concentration of elastin between all groups. A student's t-test was used to compare mRNA expression between N and WT, as well as between MP and MNP. P < 0.05 was used to indicate a statistically significant difference between groups in all cases.

Results

The ratio of total elastin to DNA (ng/ng) was significantly increased in the MP group compared to all other groups. This ratio was significantly reduced in the N group compared to MNP and WT mice. RT-PCR showed that the Col1a (p=0.03) and TIMP4 (p=0.02) expression was significantly increased in MP mice compared to MNP mice. There were no significant differences in the expression of Eln, MMP2, MMP9, TIMP3, Lox, Fbln5, and Fbn1 when MNP and MP were compared, as well as when N and WT were compared.

Interpretation of results

Loxl1 KO MP mice developed POP despite higher Col1a gene expression, and also higher elastin to DNA content, suggesting impaired ECM structure in mice with POP. Significant upregulation in TIMP4 in the same group of animals suggests positive ECM remodelling, even though the ECM that is produced is unhealthy, not being structured or functional.

Concluding message

The results of this ongoing work suggest that pathophysiologic changes in *Loxl1* KO mice, including elastin and collagen disorganization may lead to, or be triggered by POP. This could induce a compensatory regenerative response, which is aberrant and intrinsically limited in its ability to reinstate healthy ECM and thus prevent or reverse PFD. These results will inform investigation of novel biomarkers and will also shape new therapeutics based on prevention of the disease.

References

1. Chen B, Yeh, J. Alterations in connective tissue metabolism in stress incontinence and prolapse. J Urol. 2011 Nov;186(5):1768-72.
2. Lee UJ, Gustilo-Ashby AM, Daneshgari F et al. Lower urogenital tract anatomical and functional phenotype in lysyl oxidase like-1 knockout mice resembles female pelvic floor dysfunction in humans. Am J Physiol Renal Physiol. 2008 Aug;295(2):F545-55.

Disclosures

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