

AN INNOVATIVE THERAPY PREVENTS PELVIC FLOOR DISORDERS IN LYSYL OXIDASE LIKE-1 (LOXL1) KNOCKOUT (KO) MICE

Hypothesis / aims of study

Lysyl Oxidase-Like 1 (*Lox1*) knockout (KO) mice are a valuable animal model of pelvic floor disorders (PFD), including pelvic organ prolapse (POP) and stress urinary incontinence [1]. *Lox1* KO mice have aberrant connective tissue metabolism due to defective cross-linking of elastin, but the mechanism by which this deficit leads to PFD is unknown. Nonetheless, previous studies have demonstrated that the risk of PFD in *Lox1* KO mice increases with increasing parity and age [1], as in humans. Clinical studies have demonstrated decreased *Lox1* expression in genitourinary tissues of women with PFD, suggesting the clinical relevance of this model [2]. Stem-cell based therapy has the potential to treat PFD, as has been shown previously [3]. Stem cells secrete a variety of growth factors and cytokines, including those that act on connective tissue, which could supplement and/or replace deficient enzymes, potentially via a systemic rather than local effect [3]. Secretions are collected in media conditioned by culturing stem cells and are concentrated before being used as concentrated conditioned media (CCM) to treat pathologies. These secretions can, however, vary with the genetic status of individual donors. We hypothesized that CCM from bone marrow-derived mesenchymal stem cells (BM-MSC) of WT mice would have a greater protective effect against development of PFD in *Lox1* KO mice than CCM from BM-MSC of *Lox1* KO mice. The aims of this study were to assess relevant protein content of CCM from *Lox1* KO and WT mice and to determine if intra-peritoneal (IP) injection of CCM, produced from BM-MSC, extracted from genetically matched *Lox1* KO and wild type (WT) mice, has a protective effect against development of PFD in *Lox1* KO mice after multiple deliveries.

Study design, materials and methods

BM-MSCs were harvested from *Lox1* KO and wild type (WT) male mice and cultured in α minimum essential media (α -MEM) media (containing horse serum, fetal bovine serum (FBS), and antibiotic) until passage 8 to 11, when the media was replaced with antibiotic- and serum-free α -MEM media for 24h. The resultant media was collected, spun and filtered to achieve a concentration of 50X, then stored at -80°C. Concentrated control media (CM) was produced in the same manner but without being conditioned by cells. Total protein concentration in CCM was assessed by Bradford protein assay using BSA as a standard. The concentration of the proteins LOXL1, lysyl oxidase (LOX), chemokine (C-C motif) ligand 7 (CCL7), vascular endothelial growth factor (VEGF), transforming growth factor β (TGF- β), fibroblast growth factors (FGF), activin A, matrix metalloproteinases 2 (MMP2), and tissue inhibitor of MMP 2 (TIMP2) were quantified in CCM using ELISA. Seventy seven *Lox1* KO mice were bred at 8 weeks old and the male was removed when the female showed signs of a second pregnancy, for a more accurate control of multiparity. *Lox1* KO female mice were randomly distributed to receive 300 μ l of either positive CCM (CCM from WT mice), negative CCM (CCM from *Lox1* KO mice), or CM intraperitoneally within 48h of the first and second vaginal deliveries. POP was assessed weekly, as an indicator of PFD, via the mouse pelvic organ prolapse quantification (MOPQ) scoring system, a validated measurement in *Lox1* KO mice [1]. Animals with grade 2 or higher were considered to have POP. Differences in time-to-prolapse between the three groups were compared using a Kaplan-Meier survival analysis with a Dunnett-Hsu method for adjusting for multiple comparisons for the Wilcoxon test. Student's T-test was used to compare the concentration of proteins in CCM of BM-MSCs from WT and *Lox1* KO mice. $P < 0.05$ was used to indicate a statistically significant difference in both cases. Data is presented as mean \pm standard error of the mean.

Results

Lox1 KO mice treated with negative CCM showed a significant reduction of time to prolapse ($p=0.04$) compared to *Lox1* KO mice treated with control CCM. In contrast, time to prolapse in *Lox1* KO mice treated with positive CCM was not significantly different compared to CM treated mice ($p=0.14$). CM demonstrated an absence of protein content, and there was no significant difference in total protein concentration between positive ($1.08\mu\text{g}/\mu\text{l} \pm 0.32$) and negative CCM ($1.63\mu\text{g}/\mu\text{l} \pm 0.06$; $p=0.28$). Concentration of LOXL1 protein in positive CCM ranged was $0.06\text{ng}/\text{ml}/\mu\text{g}$ total protein ± 0.02 but was not detectable in negative CCM or control CM. Comparing positive and negative CCM, there were no significant differences in protein content of LOX ($p=0.5$), CCL7 ($p=0.29$), VEGF ($p=0.11$), TGF- β ($p=0.76$), FGF ($p=0.27$), activin A ($p=0.52$), TIMP2 ($p=0.59$), and MMP2 ($p=0.85$).

Interpretation of results

IP injection of negative CCM, which lacks LOXL1 protein, slowed development of POP and may prevent PFD in *Lox1* KO multiparous mice, a significantly greater effect than that of positive CCM, which contains LOXL1 protein. This suggests that CCM slows development of POP via a mechanism other than replacing the missing LOXL1 protein. However, the exact mechanism remains unknown since we were unable to demonstrate a difference in specific protein concentrations between negative and positive CCM.

Concluding message

This study suggests that a non-invasive cell-based therapy could potentially be utilized in an autologous fashion to prevent or delay development of PFD in women at high risk of PFD development. Further research is needed to clarify the mechanism of this effect.

References

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Disclosures

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