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DOES RELAXIN CONTRIBUTE TO STRESS URINARY INCONTINENCE BY ALTERING TRANSFORMING GROWTH FACTOR B (TGF-B) EXPRESSION?

Hypothesis / aims of study
Altered pelvic extracellular matrix (ECM) metabolism has been documented in women with stress urinary incontinence (SUI). TGF-β is critical to ECM since it regulates elastin and collagen synthesis and is implicated in fibrotic conditions. Latent TGF-β binding protein-1 (LTBP-1) forms a complex with latent TGF-β and targets it to the ECM. Because SUI is reported by many women during pregnancy, we sought to investigate the effect of relaxin, a peptide hormone present in high levels in pregnancy, on TGF-β1 and its binding protein (LTBP-1) expressions in vaginal fibroblasts from women with SUI compared to asymptomatic controls.

Study design, materials and methods
We obtained full-thickness, peri-urethral vaginal wall biopsies from SUI and asymptomatic premenopausal women. We excluded women with prolapse beyond the introitus. Pelvic fibroblasts from the biopsies were cultured. When confluent, these cells were stimulated with human relaxin (0-100ng/ml) for 48 hours. They were then lysed in RIPA buffer and the conditioned media collected. The remaining cell-free non-solubilized extracellular matrix was digested with 0.3 U/ml of human plasmin to release large latent TGF-β1 complexes through cleavage of LTBP-1 from the matrix. Total (acid treated samples) and active TGF-β1 in cells, supernatant, and extracellular matrix were then measured by ELISA and standardized by the amount of protein. We measured LTBP-1 expression in the supernatant by Western blot. ANOVA was used for multiple comparisons between groups.

Results
With the exception of extracellular matrix TGF-β1 expression, neither the supernatant nor fibroblasts showed a response to increasing relaxin stimulation. Total TGF-β1 in the matrix decreased in response to increasing relaxin concentrations (p = 0.001) in both SUI and control cells from women in the proliferative phase of the menstrual cycle. Active, matrix TGF-β1 expression showed the same response with increasing relaxin stimulation (p = 0.03). No consistent dose response was observed in the matrix of cells cultured from either SUI or controls in the secretory phase. The ratios of active to total TGF-β1 expression were consistently higher in the SUI cell cultures obtained during the proliferative phase compared to those from controls in both supernatant and matrix. In the cell cultures obtained during the secretory phase, this ratio was higher for the SUI group compared to controls in the supernatant. However, the ratio of active to total TGF-β1 was lower in the SUI compared to the controls in the matrix. This is consistent with increased sequestration of TGF-β1 onto the matrix in controls during the secretory phase. These differences were observed at all relaxin concentrations.

Interpretation of results
Our results indicate that relaxin may inhibit TGF-β1 sequestration into the matrix. However, cells cultured from women with SUI had a higher ratio of active to total TGF-β1 in the supernatant when stimulated with relaxin compared to those from asymptomatic women.

Concluding message
TGF-β1 has been implicated in fibrotic conditions and is critical to ECM homeostasis. Pelvic fibroblast supernatant from women with SUI consistently expressed a higher ratio of active to total TGF-β1. It is this higher ratio that may explain the altered connective tissue properties leading to SUI during pregnancy.

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