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EFFECT OF THE PELVIC FLOOR ELECTRICAL STIMULATION IN THE MUSCULAR, EXTRACELLULAR MATRIX PROTEINS AND GROWTH FACTORS IN THE URETHRA OF FEMALE RATS AFTER INDUCED TRAUMA

Hypothesis / aims of study

Damage to the components of the urethra leads to reduced intra-urethral pressure and is associated with the pathophysiology of urinary incontinence (UI). It has been shown that pelvic floor-induced trauma causes ischemia, hypoxia and structural disorganization of the urethral tissue in rodents [1, 2]. Electrical stimulation (ES) is an efficient physical therapy modality that results in increased intra-urethral pressure and it is used for UI treatment [3]. However, the molecular mechanisms underlying the benefits of ES for UI have not been investigated yet. The study of key genes and proteins in urethral tissue after injury and its regeneration process may contribute to the understanding of the pathophysiology of urethral sphincter insufficiency and can provide a solid basis for evaluating electrotherapy in urethral recovery. Therefore, in this experimental study we proposed to evaluate the effect of trauma and pelvic floor electric current in the expression of genes and proteins in the urethral muscle MYH1 (skeletal muscle fast myosin heavy chain); and those involved in the metabolism of the extracellular matrix: COL 1 (Collagen 1), COL 3 (Collagen 3), LOXL1 (Lysyl oxidase-like 1), TIMP 1 (Tissue Inhibitor of Metalloproteinase 1), MMP1 (Matrix Metalloproteinase 1); and growth factors: NGF (Nerve Growth Factor) and VEGF (Vascular Endotelial Growth Factor).

Study design, materials and methods

Pelvic floor trauma of rats was performed through 12-hour intermittent vaginal dilation with Foley catheter. ES was performed using a vaginal probe for 12 sessions of 15-min each on alternate days throughout 30 days. The urethras were extracted from four groups of rats: a) control without trauma and therapy; b) with recent induced-trauma (sacrificed 7 days post trauma), c) with late induced-trauma (sacrificed 30 days post trauma) and d) with pelvic trauma and treated with ES (sacrificed 30 days post trauma). The animals were anesthetized and had their urethras removed. The tissues were homogenized, mRNA was extracted and cDNA was obtained by reverse transcription, via RT-qPCR. A segment from each urethra was isolated, embedded in paraffin or frozen for histological and immunohistochemical analysis. Therefore we could evaluate the expression of the above cited genes and respective proteins. Kruskal-Wallis test (with Dunn's post-test) ($p<0.05$) and ANOVA test (with Tukey's post-test) ($p<0.05$) were used for statistical analysis.

Results

NGF e VEGF genes and their protein expressions were not different among the four groups. The expression of COL1 and COL3 genes were different among groups (P=0.0019 and P=0.0112): recent induced-trauma group expressions were significantly higher than control for both genes and in relation to late induced-trauma for COL1 (Fig. 1). We have observed a similar behavior regarding collagens protein expression, though not different between the groups (Fig. 2). We noted that LOXL1 mRNA expression was higher in the recent-induced trauma group compared to the control group (P=0.0041). We could not detect MMP1 expression in our samples with both methodologies. TIMP1 gene was overexpressed in the recent-induced trauma in relation to control and electrical stimulation groups (P=0.0153), without any difference regarding protein expression. The expression of MYH1 gene was significantly higher in recent and late induced-trauma groups than in control group (P=0.002). We have observed a different behavior regarding muscular protein expression: muscular immunostained area of recent induced-trauma was smaller in relation to control group (P=0.0284). Regarding the analysis of the muscular hypertrophy (evaluated by the ratio of the muscular area by urethral area) we observed that the treatment with ES had higher hypertrophy than control and late-induced trauma groups (P=0.008) (Fig. 3).

Interpretation of results

We did not observe changes in the VEGF and NGF expression as a result of the hypoxia following recent vaginal distention as expected. We postulate that those may be explained by the fast spontaneous recovery process in the animals. The increased expression of the extracellular matrix-related genes (COL1A1, COL3A1 and LOXL1) in the recent-induced trauma group corroborates our hypothesis, suggesting that in the 7th day after trauma, the urethral tissue is under a spontaneous regeneration process, but not yet completely recovered, with a reduced muscular protein. ES did not alter the expression of the genes and proteins of the extracellular matrix and growth factors, however, ES contributes for muscular hypertrophy and full recovery of the urethra, which can be noted by a higher expression of MYH1 protein the group treated with ES in relation to control and late induced-trauma groups.

Concluding message

Urethral acute recent-induced trauma causes changes in the genes responsible for the extracellular matrix metabolism and the muscular structure of the urethra. These data suggest that MYH1 protein is involved in a potential benefit of the ES in the urethral regeneration.

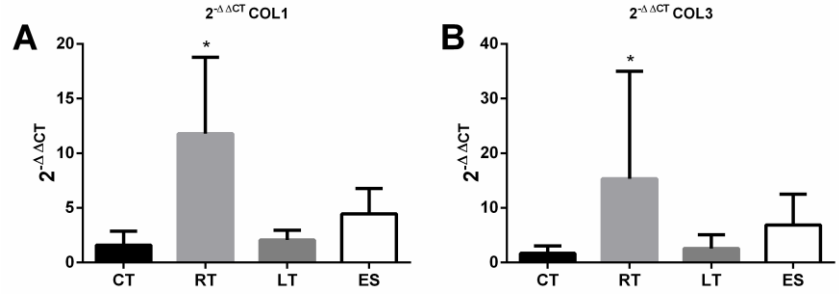

Figure 1. Gene expression of Collagen 1 (COL1, A) and Collagen 3 (COL3, B) evaluated via $2^{-\Delta\Delta Ct}$. Recent trauma group had a significantly higher expression than control group in both cases and in relation to late trauma for Collagen 1. (*:P=0.0019 and P=0.0112, respectively, Kruskal Wallis test with Dunn's post-test). CT: Control group; RT: Recent trauma group; LT: Late trauma group; ES: Pelvic trauma and treated with electrical stimulation group.

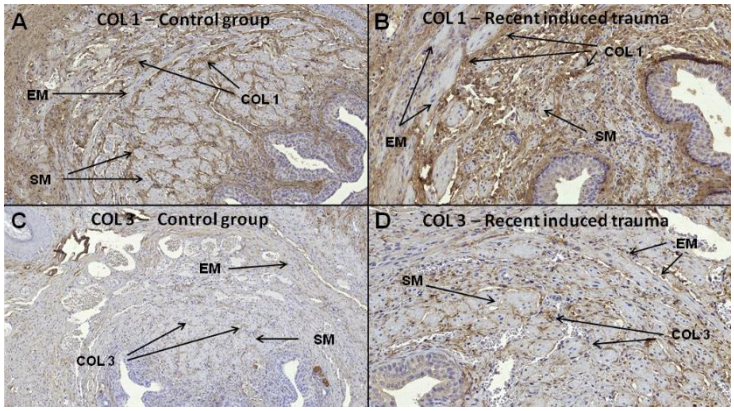

Figure 2. Protein expression of Collagen 1 (COL1, A and B, for control and recent-induced trauma, respectively) and Collagen 3 (COL3, C and D, for control and recent-induced trauma, respectively). Note the increase in the immunostained area for the recent-induced trauma groups for both COL1 and COL3. SM: Smooth muscle; EM: Skeletal muscle.

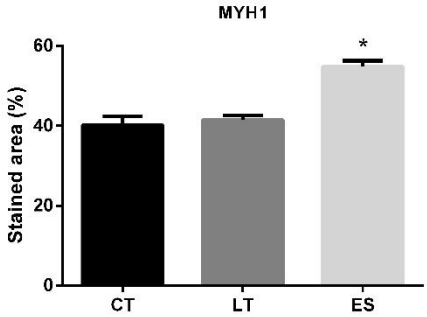

Figure 3. Gene expression of skeletal muscle fast myosin heavy chain (MYH1), which indicates the level of hypertrophy in the fast muscular fibers. Note the increased level in ES group in relation to control and late trauma groups. (*: P=0.008, One-way ANOVA with Tukey post-test). CT: Control group; RT: Recent trauma group; ES: Pelvic trauma and treated with electrical stimulation group.

References

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Disclosures

Funding: FAPESP **Clinical Trial:** No **Subjects:** ANIMAL **Species:** rat Wistar **Ethics Committee:** Ethics Committee of the Universidade Federal de Sao Paulo, CEP 1607/11