

MOLECULAR EVALUATION OF URETHRA OF FEMALE RATS AFTER INDUCED TRAUMA AND ELECTRICAL STIMULATION OF THE PELVIC FLOOR

Hypothesis / aims of study

Urinary incontinence (UI) is defined as a condition resulting in involuntary loss of urine. Damage to the components of the urethra leads to reduced intra-urethral pressure and is associated with the pathophysiology of UI. The pelvic floor-induced trauma causes ischemia, hypoxia and structural disorganization of the urethral tissues in rodents (1). Electrical stimulation is an efficient physical therapy modality that results in increased intra-urethral pressure and it is used for UI treatment. However, molecular changes in the urethra resulting from the pelvic floor injury, as well as the effect of electric current used to recover the damaged tissue in the UI treatment are still unknown. Moreover, the literature is poor regarding changes in expression of genes of structural proteins and proteins involved in tissue metabolism in urethral injury and regeneration processes (2). These genes may be involved in etiology and/or progression of UI. Thus, these findings are important for understanding the pathophysiology of urethral sphincter insufficiency and can provide a solid basis for evaluating electrotherapy as a treatment for UI. Therefore, we proposed to evaluate the effect of trauma and pelvic floor electric current in the expression of genes involved in the metabolism and structure of the urethra.

Study design, materials and methods

The urethras were extracted from four groups of rats: A) control without trauma; 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 electrical stimulation (sacrificed 30 days post trauma). The pelvic floor trauma was performed through 12-hour vaginal dilation. Electrical therapy stimulation was performed using a vaginal probe for 12 15-min sessions on alternate days throughout 30 days. The animals were anesthetized and after the extraction of the urethras they were immediately sacrificed. The urethras were homogenized, and RNA was extracted and cDNA was obtained by reverse transcription. The expression of 11 genes involved in the metabolism and structural maintenance of the urethra components were analyzed by RT-qPCR. Kruskal-Wallis test (with Dunn's post-test) ($p < 0.05$) and Pearson correlation was used for statistical analysis.

Results

Collagen 3 (COL3), collagen 1 (COL1), lysyl oxidase like 1 (LOXL1), tissue inhibitor of metalloproteinases 1 (TIMP1), myosin heavy chain of skeletal muscle 1 (MYH1), smooth muscle alpha-actin 2 (ACTA2) and smooth muscle myosin heavy chain 11 (MYH11) gene expression were significantly different among groups ($p < 0.05$). Vascular endothelial growth factor (VEGF), nerve growth factor (NGF), skeletal muscle myosin heavy chain 2 (MYH2) and Desmin gene expression were not significantly different among groups ($p > 0.05$). There was an overexpression in animal with recent induced-trauma in relation to control group for genes COL 3, COL1, LOXL1, MYH1, ACTA2, MYH11 ($p < 0.05$). There was an overexpression in animal with late induced-trauma (sacrificed 30 days post trauma) in relation to control group for genes MYH1 and MYH11 ($p < 0.05$) and there was no difference in these genes expression between pelvic trauma treated with electrical stimulation and control group ($p > 0.05$). For almost all genes there was a positive and significant correlation in their expression. We highlight the strong correlation between TIMP1 and COL1, NGF and Desmin, MYH11 and ACT2.

Interpretation of results

Overexpression of genes related to connective tissue, skeletal and smooth muscle in animal with recent induced-trauma in relation to control group may indicate the occurrence of the tissue repair process. Overexpression of MYH1 and MYH11 genes in animal with late induced-trauma (sacrificed 30 days post trauma) in relation to control group may indicate that the skeletal and smooth muscle urethra was not able to restore gene expression regular spontaneously after injury.

Concluding message

Important genes to the metabolism of collagen and skeletal and smooth muscle structures were overexpressed in urethras in animals with induced-trauma, with significant correlations among them. The electric therapy in induced-trauma animals may contribute to the restoration of most of these genes' expressions to their normal levels.

Figure 1

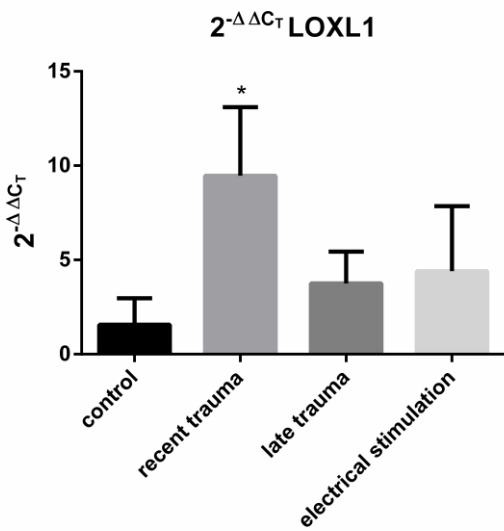


Figure 2

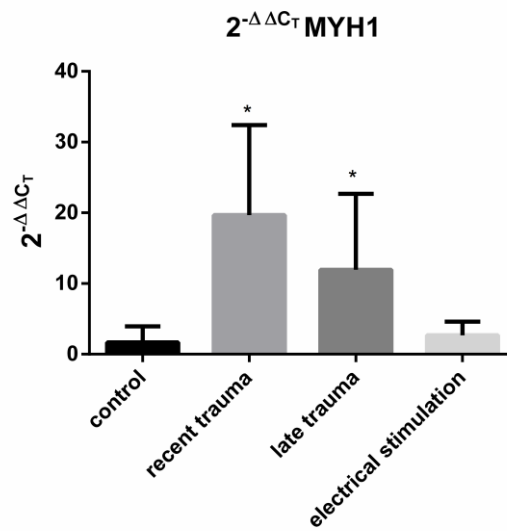
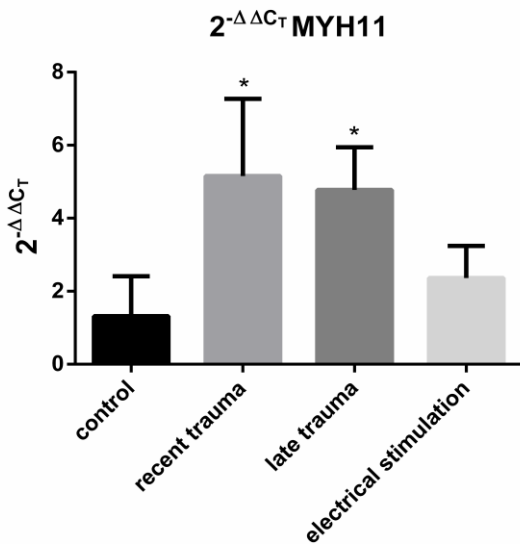


Figure 3



References

1. Cannon TW, Wojcik EM, Ferguson CL, Saraga S, Thomas C, Damaser MS. Effects of vaginal distension on urethral anatomy and function. *BJU Int*, 90(4):403-7, 2002.
2. Yiu R, Lefaucheur JP, Atala A. The regeneration process of the striated urethral sphincter involves activation of intrinsic satellite cells. *Anat Embryol*, 206(6):429-35, 2003.

Disclosures

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