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Proteomic analysis related to stress urinary incontinence following vaginal trauma in female mice

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ABSTRACT

Objective: The molecular mechanisms underlying stress urinary incontinence (SUI) are not clear. In light of the limited availability of human tissue for study, we explored the changes in the urethra of C57BL/6 mice with experimentally induced SUI.
Study design: Twelve virgin female mice were randomized into two groups: one group undergoing vaginal distension (VD) for 1 h with an 8-mm dilator, and a non-instrumented control group. Four days after VD, leak point pressures (LPP) and maximum urethral closure pressure (MUCP) were assessed in these mice under urethane (1 g/kg, i.p.) anesthesia. After measuring LPP and MUCP, the animals were sacrificed, and the urethras were removed for proteomic analysis using 2-dimensional differential gel electrophoresis (2D DIGE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) technology. Lastly, interaction between these proteins was further analyzed using MetaCore.
Results: LPP and MUCP values were significantly decreased in the 8-mm VD groups compared with the non-instrumented control group. Sixty-eight differentially expressed proteins of urethra from female mice with and without VD were identified. Of these, 19 proteins were up-regulated and 49 were down-regulated. The majority of the VD-induced proteins were involved in generation of precursor metabolites and energy, oxidation of reduction, regulation of apoptosis, and glycolysis. Myosin expression in the urethra was significantly decreased in the 8-mm VD group as compared with the control group.
Conclusions: As a model of simulated birth trauma, VD can induce SUI in female mice. Under-expression of myosin plays a plausible role in the pathogenesis of SUI following vaginal trauma.

1. Introduction

Stress urinary incontinence (SUI) is a disease that is defined as the involuntary leakage of urine under vesical stress conditions [1]. The primary etiological factor of SUI is vaginal delivery [2], usually due to combined muscular, nerve and connective tissue injury [3].

Although progress has been made in the treatment of SUI [4], our understanding of the molecular mechanisms underlying the condition is poor. Because of the limited availability of human tissue for study, animal models are an important adjunct in improving our understanding of SUI [5]. Over the last decade, animal models of SUI have increasingly been used to understand the pathogenesis of the condition [6]. Vaginal distension (VD; simulated birth trauma; vaginal trauma) [7] and pudendal nerve transection [8] have been used for inducing SUI in rats, as evidenced by lowered leak point pressures (LPP) on urodynamic testing.

Birth trauma from vaginal delivery may include denervation damage, ischemia and mechanical injuries to the muscular, neural and connective components of the lower urinary tract tissues [3,9–12]. Proteomics aiming at identification and quantification of the entire protein content (proteome) of tissue at a given time may provide insights into the mechanisms of diseases [13].

Our general goal is to understand the molecular mechanism related to SUI following vaginal trauma. Based on the relevant literature, we designed the present study with the following aims: (1) to examine LPP and maximum urethral closure pressure

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(MUCP) in C57BL/6 mice after VD; (2) to identify candidate target proteins using 2-dimensional differential gel electrophoresis (2D DIGE) and liquid chromatography–tandem mass spectrometry: (LC–MS/MS) technology; and (3) to confirm these potential target proteins using Western blot analysis, immunofluorescence staining, and immunohistochemistry staining. We expect this information to offer clues to the pathogenesis of SUI and to open additional avenues for novel research and potential therapies.

2. Materials and methods

2.1. Experimental animals and design

Twelve virgin female C57BL/6 mice, aged 6–8 weeks, were randomized into two groups: one group undergoing VD for 1 h with an 8-mm dilator (compatible with the diameter of mouse newborn head) each and a non-instrumented control group [14,15]. Two days after VD, the mice underwent suprapubic bladder tubing (SPT) placement [14–16]. Four days after VD, LPP and MUCP were assessed in these mice under urethane (1 g/kg, i.p.) anesthesia. The non-instrumented control group did not undergo VD but did undergo SPT placement and LPP measurement. After measurements, the animals were sacrificed, and the urethras were removed for proteomic and further analyses. All experimental protocols were approved by the Institutional Animal Care and Use Committee of China Medical University.

2.2. Leak point pressure measurement (LPP)

The bladder catheter was connected to both a syringe pump and a pressure transducer. Pressure and force transducer signals were amplified and digitized for computer data collection at 10 samples per second (PowerLabs, AD Instruments, Bella Vista, Australia). The mice were placed supine at the level of zero pressure while their bladders were filled with room temperature saline at 1 ml/h through the bladder catheter. If a mouse voided, the bladder was emptied manually using Crede's maneuver. The average bladder capacity of each mouse was determined after 3–5 voiding cycles. Subsequently, the LPP was measured in the following manner [14–16]. When half-bladder capacity was reached, gentle pressure with one finger was applied to the mouse's abdomen. Pressure was gently increased until urine leaked, at which time the externally applied pressure was quickly removed. The peak bladder pressure was taken as the LPP. At least three LPPs were obtained for each animal, and the mean LPP was calculated.

2.3. Urethral pressure profile (UPP)

UPP was assessed in these mice under urethane (1 g/kg, i.p.) anesthesia. The bladder catheter (PE-10 tubing, Clay Adams, Parsippany, NJ) was connected to a syringe pump with saline at 1 ml/h. The urethral catheter was connected to a pressure transducer. A withdrawal speed of 10 μm per minute was used. Pressure and force transducer signals were amplified and digitized for computer data collection at 10 samples per second (PowerLabs, ADInstruments, Bella Vista, Australia). Three successive profiles were obtained in the supine position. The urethral closure pressure (P_{close}) is the difference between the urethral pressure (P_{ure}) and the bladder pressure (P_{ves}): $P_{close} = P_{ure} - P_{ves}$ [17]. Maximum urethral pressure (MUP) and MUCP were determined from the UPP measurements taken.

2.4. Protein preparation

Frozen urethras were pulverized with a liquid nitrogen-chilled mortar and pestle. Tissue powder was then homogenized in buffer (16 mM potassium phosphate, pH 7.8, 0.12 mol/l NaCl, 1 mM ethylenediaminetetraacetic acid) containing a protease inhibitor cocktail (Complete Mini, Roche Diagnostics), and then centrifuged at 10,000 × g. The supernatant was removed, and the previous homogenization step was repeated after resuspending the remaining tissue pellet in basic buffer. After removal of the second supernatant, the remaining tissue pellet was suspended in urea buffer (6 M). The samples were centrifuged (13,000 × g for

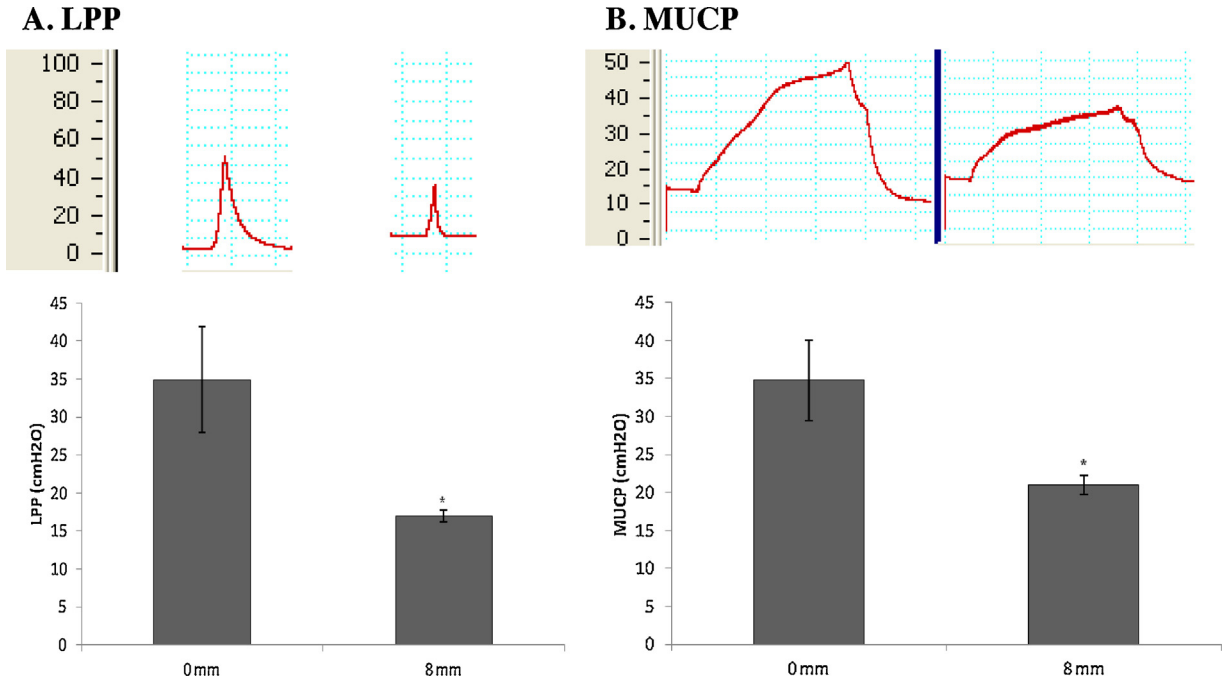

Fig. 1. LPP and MUCP values on the fourth day after vaginal distension (VD) in the different groups. Each bar represents the mean ± standard deviation of sex individual mice. *Significantly different from the value in the non-instrumented control group ($P < 0.05$).