Effects of acupuncture on abdominal leak point pressure and c-Fos expression in the brain of rats with stress urinary incontinence

In-Myong Chung\textsuperscript{a}, Youn-Sub Kim\textsuperscript{a}, Yun-Hee Sung\textsuperscript{b}, Sung-Eun Kim\textsuperscript{b}, Il-Gyu Ko\textsuperscript{b}, Mal-Soon Shin\textsuperscript{b}, Hi-Joon Park\textsuperscript{c}, Dae-Hyun Ham\textsuperscript{c}, Hye-Jung Lee\textsuperscript{c}, Ki-Jeong Kim\textsuperscript{b,d}, Sang-Won Lee\textsuperscript{b,d}, Yong-Seok Jee\textsuperscript{e}, Khae-Hawn Kim\textsuperscript{f}, Chang-Ju Kim\textsuperscript{b,∗}

\textsuperscript{a} Department of Anatomy-Meridian, College of Oriental Medicine, Kyungwon University, Seongnam 461-701, South Korea
\textsuperscript{b} Department of Physiology, College of Medicine, Kyung Hee University, #1 Hoigi-dong, Dongdaemoon-gu, Seoul 130-701, South Korea
\textsuperscript{c} Acupuncture and Meridian Science Research Center, Kyung Hee University, Hoigi-dong, Dongdaemoon-gu, Seoul 130-701, South Korea
\textsuperscript{d} Department of Physical Education, College of Education, Seoul National University, Sillim-dong, Gwanak-gu, Seoul 151-742, South Korea
\textsuperscript{e} Department of Leisure Sports, College of Science, Han Seo University, Daeung-ri, Haemi-myun, Seosam-si, Chungcheongnam-do 356-706, South Korea
\textsuperscript{f} Department of Urology, Gil Medical Center, Gachon University of Medicine and Science, Yoensu-gu, Inchon 406-799, South Korea

\textsuperscript{∗} Corresponding author. Tel.: +82 2 961 0407; fax: +82 2 964 2195.
E-mail address: changju@khu.ac.kr (C.-J. Kim).

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A B S T R A C T

Stress urinary incontinence leads to the involuntary loss of urine during abdominal strain caused by sneezing, laughing, and coughing. Acupuncture has been widely used for the treatment and prevention of a variety of diseases in traditional medicine. Acupuncture has also been used to relieve the symptoms of functional disorders of the lower urinary tract. In the present study, we investigated the effect of acupuncture at the Sanyinyijiao (SP6) acupoint on stress urinary incontinence in rats. The present results showed that abdominal leak point pressure was decreased in rats with stress urinary incontinence, while acupuncture at the SP6 acupoint significantly enhanced the abdominal leak point pressure. The expression of c-Fos in the pontine micturition center (PMC), ventrolateral periaqueductal gray (vlPAG), and medial preoptic nucleus (MPA) regions was increased by the induction of stress urinary incontinence, and acupuncture at the SP6 acupoint significantly decreased c-Fos expression in these areas. In the present study, we showed that acupuncture has therapeutic effect on the symptoms of stress urinary incontinence, and this effect of acupuncture is associated with modulation of c-Fos expression in the brain.

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[4]. A previous study found that electroacupuncture decreased c-Fos expression in the spinal cord induced by noxious stimulation of the rat bladder [11]. In the present study, we investigated the effects of acupuncture at the SP6 acupoint on stress urinary incontinence in rats using abdominal leak point pressure and immunohistochemistry for c-Fos expression.

Adult female Sprague–Dawley rats weighing 210 ± 10 g (7 weeks in age) were used. The animals were housed under controlled conditions of temperature (20 ± 2 °C) and lighting (07:00–19:00 h). The experimental procedures were performed in accordance with the animal care guidelines of National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. The animals were randomly divided into four groups (n = 8 in each group): the sham-operation group, urethrolysis-treated group, urethrolysis and acupuncture-treated group, and urethrolysis and non-acupoint acupuncture-treated group. The rats were anesthetized with Zoletil 50® anesthesia (10 mg/kg, i.p.; Virbac Laboratories, Carros, France). Urethrolysis was performed according to the previously described method [36]. In brief, after abdominal incision, the bladder and ureters were detached from the surrounding tissues, and the urethra was detached from the anterior pubic bone. In the sham-operation group, an abdominal incision was made, but the urethra was not detached. All rats were allowed 2 weeks to recover after surgery.

Abdominal leak point pressure was measured at 4 weeks after surgery in order to assess the resistance of the urethral sphincter according to the previously described method [21]. The rats were anesthetized with Zoletil 50® anesthesia (10 mg/kg, i.p.; Virbac Laboratories). All rats underwent T9 spinal cord transection to eliminate spontaneous bladder activity. After abdominal incision, an intravesical catheter was inserted and connected to a pressure transducer (Harvard Apparatus, Holliston, MA) in the dome of the bladder. All rats were mounted in the vertical position on a tilt table. Abdominal leak point pressure was then measured using Labscribe (iWorx/KB Science Inc., Dover, NH) as the peak bladder pressure for inducing urethral leakage by slow manual abdominal compression. Abdominal leak point pressure was repeatedly tested 10 times in each rat.

The animals in the acupuncture treatment groups received acupuncture stimulation once daily (at 10:00 am) for 2 weeks starting on the 14 days after surgery. For acupuncture stimulation, stainless acupuncture needles of 0.3 mm diameter were inserted bilaterally into the SP6 acupoint for the acupuncture-treated group, and into the femoral region for the non-acupoint acupuncture-treated group. After insertion, the needles were manually rotated counter-clock-wise 54 times in each leg to increase the efficiency of acupuncture and then held in place for 20 min in accordance with the previously described method [23,27].

After the abdominal leak point pressure was measured, the rats were deeply anesthetized with Zoletil 50® anesthesia (10 mg/kg, i.p.; Virbac Laboratories). They were transcardially perfused with 0.05 M phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in 0.5 M sodium phosphate buffer (PB) at pH 7.4. The brain was removed, postfixed in the same fixative overnight, and transferred into a 30% sucrose solution for cryoprotection. A freezing microtome (Leica, Nussloch, Germany) was used to collect serial coronal 40-μm sections.

For the detection of c-Fos expression, immunohistochemistry was performed according to a previously described method [22]. Free-floating sections were initially incubated in 3% H2O2 for 30 min. The sections were incubated in blocking solution (1% BSA and 10% goat serum in 0.05 M PBS) for 2 h at room temperature, then they were incubated overnight with anti-c-Fos antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C, and incubated for 1 h with biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories, Burlingame, CA) at room temperature. The sections were subsequently incubated with a Vector Elite ABC kit (Vector Laboratories) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution containing 0.05% 3,3′-diaminobenzidine (DAB) and 0.01% H2O2 in 0.05 M Tris buffer (pH 7.6) for 5 min. The sections were mounted on gelatin-coated slides and coverslipped.

To assess c-Fos expression, cell counting was performed using the Image-Pro® Plus computer-assisted image analysis system (Media Cyberbetics Inc., Silver Spring MD). The number of c-Fos-positive cells was expressed as number of cells per mm². The results are presented as the mean ± standard error of the mean (S.E.M.). The data were analyzed by one-way ANOVA followed by Duncan’s post-hoc test using SPSS. Differences were considered statistically significant at P < 0.05.

The abdominal leak point pressure is presented in Fig. 1. The abdominal leak point pressure was 50.63 ± 1.67 cmH2O in the sham-operation group, 19.22 ± 0.39 cmH2O in the urethrolysis-treated group, 33.03 ± 0.64 cmH2O in the urethrolysis and acupuncture-treated group, and 20.76 ± 2.12 cmH2O in the urethrolysis and non-acupoint acupuncture-treated group. The present results showed that urethrolysis decreased the abdominal leak point pressure, while acupuncture significantly increased the abdominal leak point pressure.

Photomicrographs of c-Fos-positive cells in the PMC region are presented in Fig. 2. The number of c-Fos-positive cells was 158.08 ± 16.86/mm² in the sham-operation group, 283.12 ± 20.12/mm² in the urethrolysis-treated group, 198.01 ± 34.94/mm² in the urethrolysis and acupuncture-treated group, and 263.47 ± 13.54/mm² in the urethrolysis and non-
acupoint acupuncture-treated group. Photomicrographs of c-Fos-positive cells in the vIPAG region are presented in Fig. 3. The number of c-Fos-positive cells was 57.26 ± 7.78/mm² in the sham-operation group, 121.06 ± 5.19/mm² in the urethrolysis-treated group, 74.49 ± 10.52/mm² in the urethrolysis and acupuncture-treated group, and 101.68 ± 3.95/mm² in the urethrolysis and non-acupoint acupuncture-treated group. Photomicrographs of c-Fos-positive cells in the MPA region are presented in Fig. 4. The number of c-Fos-positive cells in the MPA was 91.68 ± 8.05/mm² in the sham-operation group, 166.98 ± 16.45/mm² in the urethrolysis-treated group, 99.59 ± 6.97/mm² in the urethrolysis and acupuncture-treated group, and 135.66 ± 4.98/mm² in the urethrolysis and non-acupoint acupuncture-treated group.

Urinary incontinence is classified into three subtypes based on symptoms and pathologic mechanisms: urge urinary incontinence, stress urinary incontinence, and overflow urinary incontinence. Stress urinary incontinence is the most common form of urinary incontinence in women. Stress urinary incontinence results from laxity in the muscles of the pelvic floor, loss of urinary sphincter function, failure of urethral closure to prevent urine leakage from the bladder, and birth trauma [13,30].

The results of the present study showed that abdominal leak point pressure was decreased by the surgical procedure of urethrolysis and that acupuncture significantly increased abdominal leak point pressure. Bladder-to-urethral reflex activity can contribute to the maintenance of high leak point pressure during abdominal compression and increase passive intravesical pressure [24]. Conway et al. [14] reported that leak point pressure was significantly reduced by transection of somatic nerves innervating the muscles.
Fig. 3. Effects of acupuncture on the number of c-Fos-positive cells in the ventrolateral periaqueductal gray (vlPAG) region. Upper: photomicrographs of c-Fos-positive cells in the vlPAG region. The scale bar represents 50 μm. Lower: mean number of c-Fos-positive cells in each group. (A) Sham-operation group; (B) urethrolysis-treated group; (C) urethrolysis and acupuncture-treated group; (D) urethrolysis and non-acupoint acupuncture-treated group. Results are presented as the mean ± standard error mean (S.E.M.). * represents *P* < 0.05 to the sham-operation group. # represents *P* < 0.05 to the urethrolysis-treated group.

of the external urethral sphincter and pelvic floor during abdominal compression.

The present results showed that urethrolysis increased c-Fos expression in the PMC region and that acupuncture significantly decreased the urethrolysis-induced expression of c-Fos. PMC has rich afferent projections via the lumbosacral cord from the bladder and other pelvic organs [7,16]. Electrical or chemical stimulation of the PMC causes bladder contraction, the inhibition of the external urethral sphincter and relaxation of the urethra [15,19,32]. Bon et al. [9] reported that cyclophosphamide injections that caused cystitis increased the number of Fos-positive cells in the PMC.

The present results showed that urethrolysis increased c-Fos expression in the vlPAG region and that acupuncture significantly decreased the urethrolysis-induced expression of c-Fos. The vlPAG plays a critical role in regulating the micturition reflex [34]. Blockade of micturition by focal injection of cobalt chloride (CoCl₂) into the caudal vlPAG attenuated volume-evoked bladder contractions and external urethral sphincter activity [33]. Noxious stimulation on deep structures, such as the muscles, joints, and viscera significantly increased the number of c-Fos-positive neurons in the vlPAG [25,26,31]. Mitsui et al. [34] indicated that chemical bladder irritation provoked c-Fos expression in the rat vlPAG.

The present results showed that urethrolysis increased c-Fos expression in the MPA region and that acupuncture significantly decreased the urethrolysis-induced c-Fos expression. It is known that stimulation on MPA induced Fos expression within discrete regions of the pontine tegmentum: Barrington's nucleus, located ventral and medial to the rostral pole of the locus coeruleus region [37].

In urology, the application of acupuncture has been primarily limited to the modification of bladder function, especially in cases of female urethral syndrome, nocturnal enuresis, and bladder
the sham-operation group. "#" represents instability of unknown origin [11]. The present results showed that preoptic nucleus (MPA) region. Upper: Photomicrographs of c-Fos-positive cells in the medial preoptic nucleus (MPA) region. Lower: Mean number of c-Fos-positive cells in each group. (A) Sham-operation group; (B) urethrolysis-treated group; (C) urethrolysis and acupuncture-treated group; and (D) urethrolysis and non-acupoint acupuncture-treated group. Results are presented as the mean ± standard error of the mean (S.E.M.). "#" represents P<0.05 in comparison to the sham-operation group. "*" represents P<0.05 to the urethrolysis-treated group.

Fig. 4. Effects of acupuncture on the number of c-Fos-positive cells in the medial preoptic nucleus (MPA) region. Upper: Photomicrographs of c-Fos-positive cells in the MPA region. The scale bar represents 100 μm. Lower: Mean number of c-Fos-positive cells in each group. (A) Sham-operation group; (B) urethrolysis-treated group; (C) urethrolysis and acupuncture-treated group; and (D) urethrolysis and non-acupoint acupuncture-treated group. Results are presented as the mean ± standard error of the mean (S.E.M.). "#" represents P<0.05 in comparison to the sham-operation group. "*" represents P<0.05 to the urethrolysis-treated group.

instability of unknown origin [11]. The present results showed that acupuncture at the SP6 acupoint improves the symptoms of stress urinary incontinence. Philp et al. [35] reported that acupuncture at the SP6 acupoint showed therapeutic effectiveness in patients with diurnal symptoms associated with idiopathic bladder instability when compared to other non-invasive treatments. Chang et al. [11] reported that acupuncture at the SP6 acupoint could be used as a simple and effective method for treating female patients with symptoms of frequency, urgency, and dysuria.

It is known that c-Fos can be induced by stresses. In this study, we treated the rats carefully to minimize the c-Fos expression in the brain which can be induced by acupunctural stress. The increased c-Fos expression in the PMC, vPAG, and MPA induced by stress urinary incontinence demonstrated that stress urinary incontinence evokes micturition stimuli, while the suppression of c-Fos expression by acupuncture at the SP6 acupoint showed that acupuncture improved the symptoms of stress urinary incontinence. In this study, we showed that acupuncture can be used as an effective therapeutic modality to ameliorate the symptoms of urinary incontinence, and this effect of acupuncture is associated with modulation of c-Fos expression in the brain.

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