Basic Research

Effect of Treadmill Exercise on Leak-point pressure and Neuronal Activation in Brain of Rats with Stress Urinary Incontinence

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Purpose: Stress urinary incontinence (SUI) commonly occurs in women, and it causes enormous impact on quality of life. Surgery, drugs, and exercise have been recommended for the treatment of this disease. Among these exercise is also known to be effective for relieving the symptoms of SUI, however, the efficacy and underlying mechanisms of exercise on SUI are poorly understood. In the present study, we investigated the effect of treadmill exercise on abdominal leak-point pressure and neuronal activity in the medial preoptic nucleus (MPA), ventrolateral periaque ductal gray (vlPAG), and pontine micturition center (PMC) following urethrolysis in rats.

Materials and Methods: Adult female Sprague-Dawley rats, weighing 250±10 g (9 weeks old), were used in this study. After having undergone transabdominal urethrolysis to induce SUI, the rats were divided into three groups (n=6 in each group): a sham operation group, an SUI-induced group, and an SUI-induced and treadmill exercise group. The rats in the exercise group performed treadmill running for 30 min once a day starting 2 weeks after the induction of SUI and continuing for 4 weeks after surgery. For this study, determination of abdominal leak point pressure and immunohistochemistry for c-Fos in the brain were performed.

Results: Induction of transabdominal urethrolysis significantly reduced the abdominal leak point pressure, thereby contributing to the induction of SUI. In contrast, abdominal leak point pressure was significantly improved by treadmill exercise. The expression of c-Fos in the MPA, vlPAG, and PMC, the brain areas relating to micturition, was enhanced by the induction of SUI, whereas treadmill exercise significantly suppressed SUI-induced c-Fos expression, suggesting that neuronal activation in the micturition centers was suppressed by treadmill exercise.

Conclusion: The present results suggest that treadmill exercise may be an effective therapeutic modality for ameliorating the symptoms of SUI. Int Neurourol J 2010;14:141-8.

Key words: Urinary incontinence, Micturition, Treadmill test, c-Fos, Rats

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Introduction

Urinary incontinence, defined as an involuntary loss of urine, is a common health problem among women of all ages. The prevalence rate of urinary incontinence in women is between 12% and 55% worldwide [1]. This disease is associated with self-rated health, impaired quality of life, social isolation, and depressive symptoms [2].

Urinary incontinence is generally divided into four types: stress incontinence, urge incontinence, overflow incontinence, and mixed type. Stress urinary incontinence (SUI) is the most common type of urinary incontinence. The primary etiologic factor of SUI is vaginal parity, usually due to combined injuries to the muscles, nerves, and connective tissues [3,4]. Some clinicians consider SUI as a purely gross anatomical deficit that resides in the proximal urethra [5]. SUI is defined as an involuntary loss of urine secondary to the increased intraabdominal pressure that occurs by physical stresses, such as coughing, laughing, sneezing, changing to an upright posture, and climbing [6].

Generally, the micturition process involves the urinary tract and brain. Neuroanatomical tracing studies have shown that the bladder and external urethral sphincter are innervated directly or indirectly by many central nerve system regions, including the pontine micturition center (PMC), locus coeruleus, hypothalamus, preoptic area, and spinal cord [7,8]. Barrington’s nucleus is commonly referred to as the PMC or M-region. It plays an important role in the control of urinary bladder function. In addition, Barrington’s nucleus has historically been viewed as the supraspinal switching center that regulates the storage and elimination of urine [9]. The PMC is densely innervated by the medial preoptic nucleus (MPA) [10]. Two regions that maintain direct projections to the PMC are the periaqueductal gray matter (PAG) and the MPA of the hypothalamus [11]. The PAG-PMC projection is thought to take part in the micturition reflex. Neurons in the PAG regulate the micturition reflex in both animals and humans, because lesions in the PAG cause severe urinary dysfunction [11,12].

The transcription factor c-Fos is encoded by the immediately early gene (IEG) c-Fos [13]. The expression of c-Fos has been used as a marker of neuronal activity [13]. Furthermore, c-Fos expression is sometimes used as a marker for stimuli-induced changes in the metabolic activity of neurons under various conditions [14]. Stimulation of the bladder causes an increase in the number of c-Fos-immunoreactive neurons in the PAG and PMC [9,15].

Among the SUI treatments, exercise of the pelvic muscles is known to increase muscle strength and to reduce incontinent urine loss. Pelvic muscle exercise strengthens the muscles involved in closing the urethra during an increase in intra-abdominal pressure [16]. However, controversies on the efficacy of pelvic muscle exercise exist [17]. In addition, pelvic muscle exercise is known to improve symptoms rather than to be a cure, and this effect is not maintained for a long-term period [18]. Although pelvic muscle exercise has been accepted as an effective intervention for SUI, many questions on the effectiveness of treadmill exercise for the symptoms of SUI in relation to pelvic muscle exercise have been raised. Thus, evidence for the effectiveness of treadmill exercise for symptom relief in SUI is needed.

In the present study, we investigated the effects of treadmill exercise on abdominal leak point pressure and c-Fos expression in the micturition centers of the brain (MPA, vPAG, and PMC) following transabdominal urethrolysis in rats. For this study, Urodynic test for the determination of abdominal leak point pressure and c-Fos immunohistochemistry for the evaluation of neuronal activity were performed.

Materials and Methods

Animals

Adult female Sprague-Dawley rats, weighing 250±10 g (9 weeks old), were obtained from a commercial breeder (Orient Bio. Co., Seoul, Korea) for the experiment. The experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. Each animal was housed under
controlled temperature (23±2°C) and lighting (08:00 to 20:00 h) conditions and was supplied with food and water ad libitum before and after surgery. The rats were randomly divided into three groups (n=6 in each group): the sham operation group, the SUI-induced group, and the SUI-induced and treadmill exercise group.

**Surgical induction of SUI**

SUI was induced according to the previously described method [19]. The rats were anesthetized with Zoletil 50® anesthesia (10 mg/kg, i.p.; Virbac Laboratories, Carros, France). After an abdominal incision was made, the bladder and urethra were detached from the surrounding tissues and nerves, 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.

**Treadmill exercise protocol**

The rats in the exercise group performed treadmill running for 30 min once a day starting 2 weeks after the induction of SUI and continuing for 4 weeks after surgery. The exercise load consisted of running at a speed of 2 m/min for the first 5 min, 5 m/min for the next 5 min, and 8 m/min for the last 20 min, with a 0° inclination. The rats in the sham operation group and in the SUI-induced group were left on the treadmill without running for the same period as the exercise group.

**Determination of abdominal leak point pressure**

At 6 weeks after surgery, we examined abdominal leak point pressure. The rats were anesthetized with Zoletil 50® anesthesia (10 mg/kg, i.p.; Virbac Laboratories). All rats underwent T11 spinal cord transection to eliminate spontaneous bladder activity. After making an abdominal incision, we inserted an intravesical catheter connected to a pressure transducer (Harvard Apparatus Inc., Holliston, MA, USA) in the dome of the bladder. We measured the abdominal leak point pressure by using a Labscribe (iWork/CB Science, Inc., Dover, NH, USA).

All rats were mounted on a tilt table in a vertical position. Saline at room temperature was infused through the catheter, and the maximal bladder capacity was determined when the first drop of urine appeared at the urethral meatus. The bladder was manually emptied by squeezing and was then filled to one-third capacity with saline. Then, the abdominal leak point pressure was determined as the peak bladder pressure inducing leakage of urine at the urethral meatus by means of manual abdominal compression. In this way, the abdominal leak point pressure was determined 10 times for each rat.

**Tissue preparation**

After tissue preparation of the urethra, the rats were transcardially perfused with 50 mM phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in 100 mM 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. Serial coronal sections 40 μm thick were made with a freezing microtome (Leica, Nussloch, Germany). PMC was selected from the region spanning from Bregma -9.68 to -9.80 mm, vILPAG was selected from the region spanning from Bregma -7.64 to -8.00 mm, and MPA was selected from the region spanning from Bregma -0.26 to 0.80 mm. Ten sections on average in each region were collected from each rat.

**c-Fos immunohistochemistry**

Free-floating tissue sections were incubated overnight with rabbit anti-c-Fos antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at a dilution of 1:1000, and the sections were then incubated for 1 h with biotinylated anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA, USA). The sections were subsequently incubated with avidin-biotin-peroxidase complex (Vector Laboratories) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution consisting of 0.05% 3,3-diaminobenzidine (DAB) and 0.01% H2O2 in 50 mM Tris-buffer (pH 7.6) for approximately 3 min. The sections were then washed three times with PBS and mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature, and coverslips were mounted by using Permount®.
Data analysis

To assess c-Fos expression in the MPA, vIPAG, and PMC, cell counting was performed by using the Image-Pro® Plus computer-assisted image analysis system (Media Cyberbetics Inc., Silver Spring, MD, USA) attached to a light microscope (Olympus, Tokyo, Japan). The number of c-Fos-positive neurons was counted hemilaterally. Statistical analysis was performed by using one-way ANOVA followed by Duncan's post-hoc test, and the results are expressed as the mean±standard error of the mean (S.E.M.). Significance was set as p<0.05.

Results

Effect of treadmill exercise on abdominal leak point pressure

The abdominal leak point pressure is presented in fig. 1. The abdominal leak point pressure was 32.68±0.57 cmH₂O in the sham operation group, 12.74±1.14 cmH₂O in the SUI-induced group, and 24.84±0.91 cmH₂O in the SUI-induced and treadmill exercise group.

These results showed that induction of SUI by transabdominal urethrolysis decreased the abdominal leak point pressure compared with the sham operation group (p<0.05), whereas treadmill exercise increased the abdominal leak point pressure reduced by transabdominal urethrolysis (p<0.05).

Effect of treadmill exercise on the number of c-Fos-positive cells in the MPA

Photomicrographs of c-Fos-positive cells in the MPA are presented in fig. 2. The number of c-Fos-positive cells was 78.39±5.13/mm² in the sham operation group, 225.71±15.74/mm² in the SUI-induced group, and 161.07±10.87/mm² in the SUI-induced and treadmill exercise group.

These results showed that induction of SUI by transabdominal urethrolysis increased the c-Fos expression in the MPA compared with the sham operation group (p<0.05), whereas treadmill exercise suppressed the c-Fos expression in the MPA increased by transabdominal urethrolysis (p<0.05).

Effect of treadmill exercise on the number of c-Fos-positive cells in the vIPAG

Photomicrographs of c-Fos-positive cells in the vIPAG are presented in fig. 3. The number of c-Fos-positive cells was 128.89±14.49/mm² in the sham operation group, 638.25±25.27/mm² in the SUI-induced group, and 372.21±21.00/mm² in the SUI-induced and treadmill exercise group.

These results showed that induction of SUI by transabdominal urethrolysis increased the c-Fos expression in the vIPAG compared with the sham operation group (p<0.05), whereas treadmill exercise suppressed the c-Fos expression in the vIPAG increased by transabdominal urethrolysis (p<0.05).
Effect of treadmill exercise on the number of c-Fos-positive cells in the PMC

Photomicrographs of c-Fos-positive cells in the PMC are presented in fig. 4. The number of c-Fos-positive cells was 70.70±5.72/mm² in the sham operation group, 330.47±15.13/mm² in the SUI-induced group, and 257.67±10.23/mm² in the SUI-induced and treadmill exercise group.

These results showed that induction of SUI by transabdominal urethrolysis increased the c-Fos expression in the PMC compared with the sham operation group (p<0.05), whereas treadmill exercise suppressed the c-Fos expression in the PMC increased by transabdominal urethrolysis (p<0.05).

Discussion

Transabdominal urethrolysis is a reliable and long-lasting method for inducing SUI compared with existing models of SUI [20]. In the present study, we measured the abdominal leak point pressure following transabdominal urethrolysis to confirm the induction of SUI. Abdominal leak point pressure is a test assessing the function of the urethra. Maximum urethral closure pressure and leak point pressure have been used to determine the severity of incontinence, the guidelines for therapy, and the evaluation of treatment outcomes [5,21]. The reflex activity of the bladder
to urethra contributes to the maintenance of high leak point pressure during abdominal compression and increases passive intravesical pressure [22]. Abdominal leak point pressure has been used for studies of SUI, and decreased abdominal leak point pressure is observed after the induction of SUI [5,19,23].

In the present study, the induction of SUI by transabdominal urethrolysis resulted in a decrease in the abdominal leak point pressure compared with the sham operation group. These results indicate that SUI induction by transabdominal urethrolysis decreases the abdominal leak point pressure in rats.

Activation of PMC neurons induces bladder contraction and relaxation of the bladder neck and external urethral sphincter, resulting in micturition [24]. Two regions associated with the PMC are the PAG and the MPA in the hypothalamus [7]. During normal micturition, activation of the bladder stretch receptor stimulates the PAG, and then the activated PAG stimulates the PMC. The activated PMC in turn initiates complete synergic micturition responses via the excitation of parasympathetic bladder motor neurons in the sacral part of the spinal cord with the inhibition of the bladder sphincter motor neurons [25]. In previous studies, various stimulations of the lower urinary tract caused changes in central pathways, such as the PMC, PAG, MPA, and spinal cord [15]. It was also reported that transabdominal urethrolysis enhanced c-Fos expression in the PMC and vIPAG, the brain areas related to micturition [9].

Our results also showed that the expression of c-Fos in the MPA, vIPAG, and PMC was significantly increased after transabdominal urethrolysis. The present results indicate that transabdominal urethrolysis increases neuronal activity in the MPA, vIPAG, and PMC. It can be inferred that loss of supportive tissues or induction of smooth muscle atrophy by transabdominal urethrolysis might strongly stimulate the micturition-related brain areas. In our previous study, transabdominal urethrolysis-induced SUI increased c-Fos expression in the MPA, vIPAG, and PMC [26].

Treadmill exercise enhances neuronal plasticity and changes the transcription levels of various genes, resulting in increased neuronal activity and synaptic remodeling [27]. Following a bout of exercise, adaptive and protective changes in the expression of early genes occurs in several brain areas [28]. It has been hypothesized that induction of immediate early genes, such as c-Fos, initiates changes in gene expression in muscle and brain during exercise adaptation [29]. Among the causes of the various forms of SUI, the primary causes of weakened bladder and pelvic muscles are childbirth and prostate surgery. Additional contributing factors include obesity, diabetes, specific medications, and urinary tract infections [30]. Aerobic exercise including running and walking is well-known to facilitate recovery and improvement from the functional loss and

**Figure 4.** Effect of treadmill exercise on c-Fos expression in the pontine micturition center (PMC). Upper panel: Photomicrographs of c-Fos-positive cells in the PMC. The sections were stained for c-Fos-like immunoreactivity (brown). The scale bar represents 200 μm. Lower panel: The mean number of c-Fos-positive cells in each group. (A) Sham operation group, (B) SUI-induced group, (C) SUI-induced and treadmill exercise group. The results are presented as the mean±standard error of the mean (S.E.M.). *represents p<0.05 compared to sham operation group. #represents p<0.05 compared to SUI-induction group.
muscle atrophy, and this type of exercise is effective for reducing body fat in obese people. Treadmill exercise enhanced contractility in response to either agonists or depolarization in smooth muscles [31]. For this reason, treadmill exercise could improve abdominal leak point pressure. Strengthening of the muscles and tissues by exercise may relieve the symptoms of SUI. Suppression of c-Fos expression in the MPA, vIPAG, and PMC was accompanied with symptom improvement of SUI [26].

In this study, we evaluated the effect of treadmill exercise on SUI-induced abdominal leak point pressure by use of Urodynic analysis. Transabdominal urethrolisis significantly reduced the abdominal leak point pressure, thereby contributing to the induction of SUI; in contrast, abdominal leak point pressure was significantly improved by treadmill exercise. In addition, treadmill exercise significantly suppressed the SUI-induced enhancement of c-Fos expressions in the MPA, vIPAG, and PMC.

Conclusions

Treadmill exercise might act as a supplement for the loss of supportive tissues or hinder atrophy of the intrinsic sphincter. The supply of supportive tissues or the delay of smooth muscle atrophy by treadmill exercise could increase the abdominal leak point pressure and then suppress the activation of the MPA, vIPAG, and PMC, resulting in the amelioration of SUI symptoms. Our present results suggest that treadmill exercise may be used as an effective therapeutic modality to ameliorate the symptoms of SUI.

Conflicts of Interest:

The authors have nothing to disclose.

References

15) Saban MR, Nguyen NB, Hammond TG, Saban R. Gene expression profiling of mouse bladder inflammatory responses to LPS, substance P, and an-
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