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Effects of low-intensity extracorporeal shock wave on bladder and urethral dysfunction in spinal cord injured rats

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Abstract

Purpose To investigate the effects of low-intensity extracorporeal shock wave therapy (LiESWT) on bladder and urethral dysfunction with detrusor overactivity and detrusor sphincter dyssynergia (DSD) resulting from spinal cord injury (SCI).

Methods At 3 weeks after Th9 spinal cord transection, LiESWT was performed on the bladder and urethra of adult female Sprague Dawley rats with 300 shots of 2 Hz and an energy flux density of 0.12 mJ/mm^2, repeated four times every 3 days, totaling 1200 shots. Six weeks postoperatively, a single cystometrogram (CMG) and an external urethral sphincter electromyogram (EUS-EMG) were simultaneously recorded in awake animals, followed by histological evaluation.

Results Voiding efficiency significantly improved in the LiESWT group (71.2%) compared to that in the control group (51.8%). The reduced EUS activity ratio during voiding (duration of reduced EUS activity during voiding/EUS contraction duration with voiding + duration of reduced EUS activity during voiding) was significantly higher in the LiESWT group (66.9%) compared to the control group (46.3%). Immunohistochemical examination revealed that fibrosis in the urethral muscle layer was reduced, and S-100 stained-positive area, a Schwann cell marker, was significantly increased in the urethra of the LiESWT group.

Conclusion LiESWT targeting the urethra after SCI can restore the EUS-EMG tonic activity during voiding, thereby partially ameliorating DSD. Therefore, LiESWT is a promising approach for treating bladder and urethral dysfunction following SCI.

Keywords Spinal cord injury (SCI) · Detrusor sphincter dyssynergia (DSD) · Low-intensity extracorporeal shock wave therapy (LiESWT) · Urethra · External urethral sphincter-electromyogram (EUS-EMG) · Cystometrogram (CMG)

Introduction

Micturition is highly regulated by the central and peripheral nervous systems through sympathetic, parasympathetic, and somatic nerves. During the storage phase, sympathetic nerves originating from the thoracolumbar spinal cord are excited to relax the bladder smooth muscles and contract the urethral smooth muscles, whereas the stimulation of somatic nerves from Onuf's nucleus in the sacral spinal cord results in the contraction of the external urethral sphincter (EUS). During the voiding phase, the central nervous system, including the pontine micturition center, excites the parasympathetic nerves and inhibits sympathetic and somatic nerves [1, 2]. However, the coordinated action of the bladder and urethra is completely arrested following supranuclear spinal cord injury (SCI).

After SCI, the voiding reflex disappears causing acute urinary retention. In the chronic phase, the spinal voiding reflex emerges, inducing detrusor overactivity (DO) and detrusor sphincter dyssynergia (DSD) through the activation of silent C-fiber bladder afferents [3]. However, this irreversible condition results in increased post-void residual volume [4, 5], urinary tract infection, hydronephrosis, and renal function deterioration [1] Intermittent catheterization can prevent urinary disturbances [6]; however, an alternative and effective treatment for SCI has not been established.

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Various animal studies have been conducted in an attempt to treat bladder and urethral dysfunction following SCI, including intrathecal baclofen [7, 8], bladder wall injections of botulinum toxin A [9], and administration of anti-brain-derived neurotrophic factor antibodies [10]. However, the effects of these treatments are limited and they are relatively invasive. Low-intensity extracorporeal shock wave therapy (LiESWT) has anti-inflammatory effects and promotes angiogenesis, and it is already used as a simple, minimally invasive treatment method with an option for the treatment of orthopedics, angina pectoris, and erectile dysfunction [11, 12]. Moreover, LiESWT promotes tissue regeneration in the vagina and urethra, ameliorates stress urinary incontinence in rats [13], and restores damaged nerve function in diabetic rats [14]. Moreover, a recent review mentioned that LiESWT could be involved in nerve regeneration, even in a spinal cord contusion model, through the enhancement of neurotrophic factors and the activation of the neuroprotective signal pathway [15]. Thus, we hypothesized that LiESWT could reduce DSD after SCI, characterized by dramatic neurological changes.

Therefore, we aimed to elucidate the effects of LiESWT on bladder and urethral dysfunction with DO and DSD after SCI. Accordingly, we applied LiESWT to the lower abdomen of rats with chronic SCI and assessed its effects via cystometrography (CMG) and EUS electromyography (EUS-EMG), as well as histological studies of the bladder and urethra.

Materials and methods

Animals

All experimental procedures were performed using a protocol (A2021078) approved by the Institutional Animal Care and Use Committee of University of the Ryukyus, in compliance with the ARRIVE guidelines (https://arriveguidelines.org/).

Spinal cord injury (SCI) model

Twenty-one female Sprague Dawley rats (6 weeks old, weighing 140–180 g) were used in this study. The EUS-EMG activity was compared between four normal rats and SCI rats. SCI was induced in rats by Th9 transection under anesthesia with 2% isoflurane and pentobarbital (30 mg/kg subcutaneously) for analgesics on the day. The dura mater was opened, the Th9-Th10 spinal cord was removed completely using a scalpel, and a sterile sponge (LTL Pharma Co., Ltd. Tokyo, JPN) was placed between the cut ends of the spinal cord. The muscle and skin layers were closed. Postoperatively, cephazolin (100 mg/kg) was injected subcutaneously for 5 days. The bladders of spinalized rats were emptied by abdominal compression two or three times a day for 14 days until reflex voiding was restored postoperatively. We warmed animals on the day after spinalization to minimize pain; the bed was changed in the cage daily, and cream ointment was applied to the lesion on the bedsore as needed [16]. Rats with induced spinal injury were divided into two groups: the LiESWT group (n = 9) and an untreated control group (n = 8). At the end of the experiment, rats were euthanized with pentobarbital (100 mg/kg peritoneally) then the bladders were removed and weighed.

LiESWT technique

In the LiESWT group, voiding was induced in awake animals after tail pinching and Crede maneuver to reduce bladder distention. Thereafter, LiESWT was performed at 3 weeks after spinal cord transection with each 300 shots of 2 Hz and an energy flux density of 0.12 mJ/mm^2, repeated four times every 3 days, i.e., 1200 shots in total, using the ED-1000 device (Medispec, Ltd., Yehud, ISR) under isoflurane anesthesia, based on our recent acute model [16] and preliminary experiments. The shockwave applicator was placed on a gel pad (Yosojima Proceed Co., Ltd. Hyogo, JPN) on the shaved skin of the rat's lower abdomen using ultrasound gel, and the angle was adjusted toward the pelvic floor so that the targeting shock wave was involved in most of the urethra (Fig. 1).

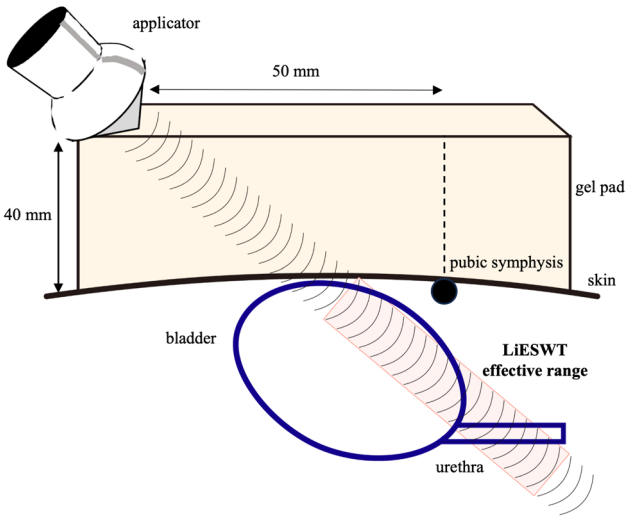

Fig. 1 Illustration of LiESWT technique. The ED-1000 probe has a therapeutic effect in a range of 9 mm in diameter and 135 mm in depth from the tip, of which a particularly effective range exists. In this study, the gel pad was doubled (40 mm) and the probe position and angle were adjusted from 50 mm cephalad from the pubic symphysis to the pelvic floor so that more urethra is in the most effective range. *LiESWT* low-intensity extracorporeal shock wave therapy

Cystometrogram (CMG) and external urethral sphincter electromyogram (EUS-EMG)

Six weeks after spinal cord transection, the rats were anesthetized with 2% isoflurane, and the abdomen was opened at the midline. Both ureters were cut, and the distal end was subjected to ligature. A polyethylene catheter (PE-50; Intramedic, Becton, Dickinson and Company, NJ, USA) was inserted into the bladder dome. After abdominal closure, rats were restrained (Universal Rat Restrainer; Braintree Scientific, Inc., MA, USA) and allowed to rest in the dark for one hour. Rats were subjected to CMG while awake. The intravesical catheter was connected to a pressure transducer and an infusion pump via a three-way valve. Saline was infused at a rate of 3.0 mL/h to induce repetitive bladder contractions and bladder activity was monitored. PowerLab (ADInstruments, Pty. Ltd., New South Wales, AUS) was used for data acquisition. Single CMG was performed to evaluate and compare the voided volume, amplitude of voiding pressure, bladder contraction duration during voiding, and number and amplitude of non-voiding bladder contractions (NVCs) between both groups. NVCs were defined as rhythmic intravesical pressure increases greater than 7 cm H_2O from the baseline pressure without voiding, and the number and amplitude of NVCs were measured 5 min before micturition. Saline volume from the urethral meatus was collected and measured to determine the voided volume. After each void, bladder infusion was stopped, and the intravesical solution was retrieved through the catheter by gravity, the volume of which was considered the post-void residual volume. The voiding efficiency (VE) was calculated using the formula: VE (%) = [voided volume/(voided volume + post-void residual volume) × 100].

EUS-EMG activity was recorded using two 500 µm polyurethane-coated stainless needle electrodes (Bio Research Center Co., Ltd. Aichi, JPN). These were inserted on both sides of the EUS device transperitoneally and connected to a computer system with an analog-to-digital converter (PowerLab). EUS-EMG data were amplified and digitized using a computer system. In SCI rats, EUS-EMG was performed to assess the changes in DSD as EUS contraction duration with voiding. Improvement in DSD was evaluated as the reduced EUS activity ratio during voiding (duration of reduced EUS activity during voiding/ EUS contraction duration with voiding + duration of reduced EUS activity during voiding) (Fig. 2). In addition, EUS-EMG was compared in normal rats (n = 4) and SCI rats.

Bladder and urethra morphology

The bladder and urethra were removed at the end of the experiment to determine the histological changes caused by LiESWT. The collected bladder and urethra were fixed in

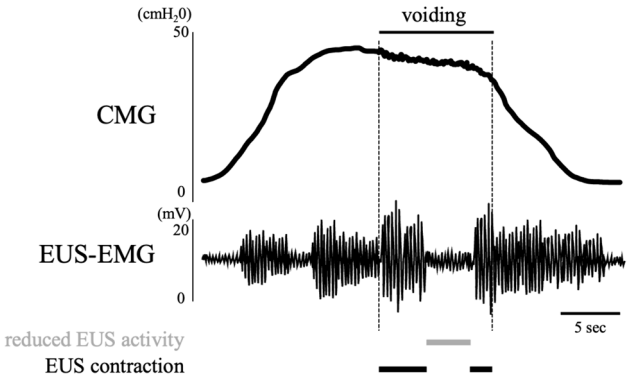

Fig. 2 Illustration of cystometrogram (CMG) and external urethral sphincter electromyogram (EUS-EMG) during voiding bladder contraction. EUS-EMG during voiding shows EUS contraction with tonic activity (i.e., detrusor sphincter dyssynergia) or reduced EUS activity

4% paraformaldehyde for 24 h, and 4-micrometer-thick sections were cut using a microtome from paraffin-embedded specimens and thawed on gelatin-coated slides. Following deparaffinization, hematoxylin and eosin (H&E) staining (HAE-1-1FU, ScyTek Laboratories Inc., UT, USA) and Masson's trichrome staining (TRM-1-1FU, ScyTek Laboratories Inc. Lcc, UT, USA) were performed according to standard protocols. In Masson's trichrome staining, three randomly selected fields of view (objective × 10 and × 20, respectively) were analyzed to quantify the fibrosis area. The percentage of the integrated area of fibrosis in each section was calculated using FIJI ImageJ software (ver. ImageJ 1.53q, Java 1.8.0_322 [64 bit], https://imagej.net/Fiji/Download).

For S-100, a Schwann cell marker, immunohistochemical examination was performed. The sections were then incubated overnight at 4 °C with rabbit primary antibody against S-100 (1:2000, GTX48819, GeneTex, Irvine, CA, USA). Thereafter, the sections were incubated with horse anti-rabbit antibodies for 30 min. The reaction sites were stained with diaminobenzidine (DAB) chromogenic agent until the desired staining intensity was achieved, and then sections were counterstained with hematoxylin. The area of the S-100 stain-positive range for each section was calculated using FIJI ImageJ software.

Statistical analysis

Data were analyzed using GraphPad Prism Version 8.4.3 (GraphPad Software Inc, CA, USA) and expressed as mean ± standard error (SE) for normally distributed data or medians and quartiles for non-normally distributed data. Unpaired Student's t-test was used for parametric data. Non-parametric data between-group comparisons were performed using the Mann–Whitney U test (Jmp Pro, version 15.0.0

(SAS Institute, NC, USA). Differences between groups were considered significant at $p < 0.05$.

Results

Comparisons of body and bladder weights between SCI control and SCI LiESWT groups

The initial body weight was not significantly different in the SCI control and SCI LiESWT groups (163.1 ± 4.7 g vs. 167.0 ± 2.9 g, p = 0.53). However, the final body weight was significantly higher in the SCI LiESWT group than in the SCI control group (284.3 ± 8.4 g vs. 257.1 ± 9.9 g, p = 0.043). There were no differences in bladder weight between the SCI control and SCI LiESWT groups (0.39 (0.31–0.41) g vs. 0.32 (0.28–0.35) g, p = 0.66).

Experiment 1: comparison of CMG between SCI control and SCI LiESWT groups

No significant differences were found regarding the amplitude of voiding pressure (53.0 ± 6.8 cmH_2O and 44.8 ± 4.2 cmH_2O, p = 0.34), the amplitude of NVC (21.9 ± 3.0 cmH_2O and 20.1 ± 2.3 cmH_2O, p = 0.63), the number of NVCs (7.8 (6.3–11.5)/5 min and 7.0 (6.7–8.0)/5 min, p = 0.66, Fig. 3), the single voided volume (0.78 ± 0.19 mL and 1.09 ± 0.22 mL, p = 0.31), or the post-void residual volume (1.18 ± 0.50 mL and 0.91 ± 0.48 mL, p = 0.63) between the SCI control and SCI LiESWT groups, respectively. However, the VE was significantly higher in the SCI LiESWT group (71.2 ± 6.6%) than in the SCI control group (51.8 ± 6.7%, p = 0.034, Table 1).

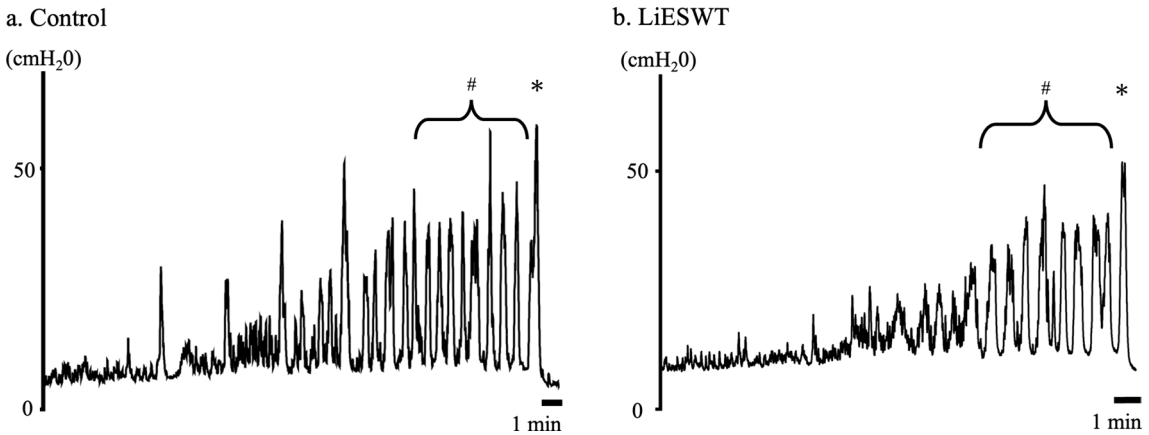

Fig. 3 Records of intravesical pressure from the start of saline infusion to voiding (*indicates a voiding contraction) in SCI rats treated without LiESWT (**a**) or with LiESWT (**b**). **a** Prominent NVCs (NVCs during 5 min are expressed as #) were observed early after the start of saline infusion. **b** NVCs gradually appeared more frequently a few minutes before voiding. *SCI* spinal cord injury, *LiESWT* low-intensity extracorporeal shock wave therapy, *NVC* non-voiding bladder contraction

Table 1 Intravesical parameters in cystometrogram in the SCI control and SCI LiESWT groups

	SCI control (n = 8)	SCI LiESWT (n = 9)	p-value
Amplitude of voiding pressure (cmH_2O)	53.0 ± 6.8	44.8 ± 4.2	0.34
Amplitude of NVC (cmH_2O)	21.9 ± 3.0	20.1 ± 2.3	0.63
Number of NVCs	7.8 (6.3–11.5)	7.0 (6.7–8.0)	0.66
Voided volume (mL)	0.78 ± 0.19	1.09 ± 0.22	0.31
Post-void residual volume (mL)	1.18 ± 0.50	0.91 ± 0.48	0.63
Voiding efficiency (%)	51.8 ± 6.7	71.2 ± 6.6	**0.034**

Bold value indicates statistically significant (p-value < 0.05) than SCI control

Values are presented as mean ± standard error (SE) or median (interquartile range)

SCI spinal cord injury, *LiESWT* low-intensity extracorporeal shock wave therapy, *NVC* non-voiding bladder contraction

Experiment 2: comparisons of EUS-EMG between SCI control and SCI LiESWT groups

In normal rats, EUS-EMG indicated bursting during voiding at 97%; however, in SCI rats, bursting activity was reduced at 4% during voiding ($p < 0.001$ versus the normal group) instead of replacement by the EUS tonic activity (Fig. 4, Table 2). There was no significant difference in the bursting activity ratio during voiding between the SCI control group (4.0 ± 3.0%) and SCI LiESWT group (10.6 ± 7.5%, $p = 0.43$). Simultaneous CMG and EUS-EMG recordings showed no significant differences in the duration of reduced EUS activity during voiding between the SCI control group (4.32 ± 0.59 s) and the SCI LiESWT group (5.05 ± 0.59 s, $p = 0.42$). However, EUS contraction duration with voiding was significantly shorter (2.47 ± 0.50 s compared to 5.17 ± 0.78 s, $p = 0.013$), and reduced EUS activity ratio during voiding was significantly higher (66.9 ± 5.1% compared to 46.3 ± 5.1%, $p = 0.017$) in the SCI LiESWT group than in the SCI control group (Table 2, Fig. 4).

Bladder and urethra morphology

As for the bladder, no significant structural differences could be identified upon H&E staining, in addition, there was no significant difference in fibrosis in Masson's trichrome between the SCI control (28.1 ± 1.8%) and SCI LiESWT groups (24.3 ± 1.7%, $p = 0.14$, Fig. 5). In the urethra, H&E staining showed no difference in hyperplasia of the urothelium, but the SCI LiESWT group showed significantly lower fibrosis in Masson's trichrome (35.8 ± 1.0%) than the SCI control group (41.9 ± 0.5%, $p = 0.023$, Fig. 5). In S-100 immunostaining of the urethra, the positive area was significantly increased in the SCI LiESWT group than in the SCI control group (3.0 ± 0.6% vs, 1.3 ± 0.2%, $p = 0.016$, Fig. 5). The S-100 immunostaining of the bladder revealed no significant differences between the SCI LiESWT and SCI control groups (data not shown).

Discussion

The results of the present study indicate that the SCI LiESWT group showed: (1) higher voiding efficiency, (2) higher reduced EUS-EMG activity during voiding, (3) a more prominent effect on the urethra, indicated by the lower fibrosis rate in the muscle layer of the urethra, (4) increased Schwann cell markers as indicated by increased S-100 immunostaining positive areas in the urethra, and (5) higher voiding efficiency with lesser chance of urinary tract infection, which affected general conditions, as indicated by the significant increase in body weight, than those in the control group. These results indicate that applying LiESWT to the pelvis of SCI rats may promote EUS relaxation and restore the urethral nervous system, improving urinary conditions, primarily DSD.

In the chronic phase of SCI, the bladder becomes hyperreflexic due to an overactive spinal micturition reflex pathway, and the loss of coordination between the detrusor muscle and EUS leads to DSD and decreased VE [4, 5, 17, 18]. In the present study, the amplitude of voiding pressure was relatively lower in the LiESWT group than in the control group. As DSD results in less efficient EUS relaxation and higher voiding pressure [17], LiESWT may have affected EUS function. In fact, applying LiESWT to the urethra of rats with SCI increased the reduced EUS-EMG activity ratio during voiding. In rats and dogs with a normal spiral cord, tonic activity prior to the onset of voiding and bursting activity with clusters of high-frequency spikes during voiding were detected using EUS-EMG as shown in Fig. 4. This bursting activity results in rhythmic contraction and relaxation of the EUS and contributes to synergic urethral pumping activity [5]. In humans, EUS-EMG during voiding is silenced and the EUS relaxes [19] which is relatively similar to that in female mice [20]. The action of EUS during urination differs between species and sexes, and EUS bursting occurs during voiding bladder contractions in spinal intact and SCI rats [5, 19, 20]. In the present study, the rhythmic bursting during voiding was observed along with significant reduction to 4% in SCI rats instead of replacement by EUS tonic activity. However, reduced EUS-EMG activity ratio during voiding (duration of reduced EUS activity during voiding/EUS contraction duration with voiding + duration of reduced EUS activity during voiding) was higher in the LiESWT group. LiESWT may have enhanced the cooperation of the bladder and urethral sphincter and partially contributed to improving DSD and VE. This is the first report to demonstrate the effect of LiESWT on DSD in SCI models.

In the chronic phase of SCI, the increase in NVCs can be prevented by capsaicin pretreatment; thus, C-fiber bladder afferent nerves are involved in NVCs in SCI [3, 21] However, the present study found no differences in NVCs between the control and LiESWT groups. LiESWT improved DSD and reduced the fibrosis rate in the muscle layer, particularly in the urethra. These findings indicate that applying LiESWT to the pelvis may have affected the urethra rather than the bladder. Tissue fibrosis is caused by excessive deposition of extracellular matrix due to myofibroblast proliferation associated with chronic inflammation [22]. In rabbits, extracorporeal shock wave therapy reduced the number of collagen bundles by suppressing myofibroblast expression [23]. A previous study has reported an increase in the number of progenitor cells in the urethral smooth muscle layer of vaginal balloon-dilated rats treated with LiESWT, resulting in dramatic recovery of the smooth and striated muscle mass [13]. In this study, there was no change in fibrosis in the

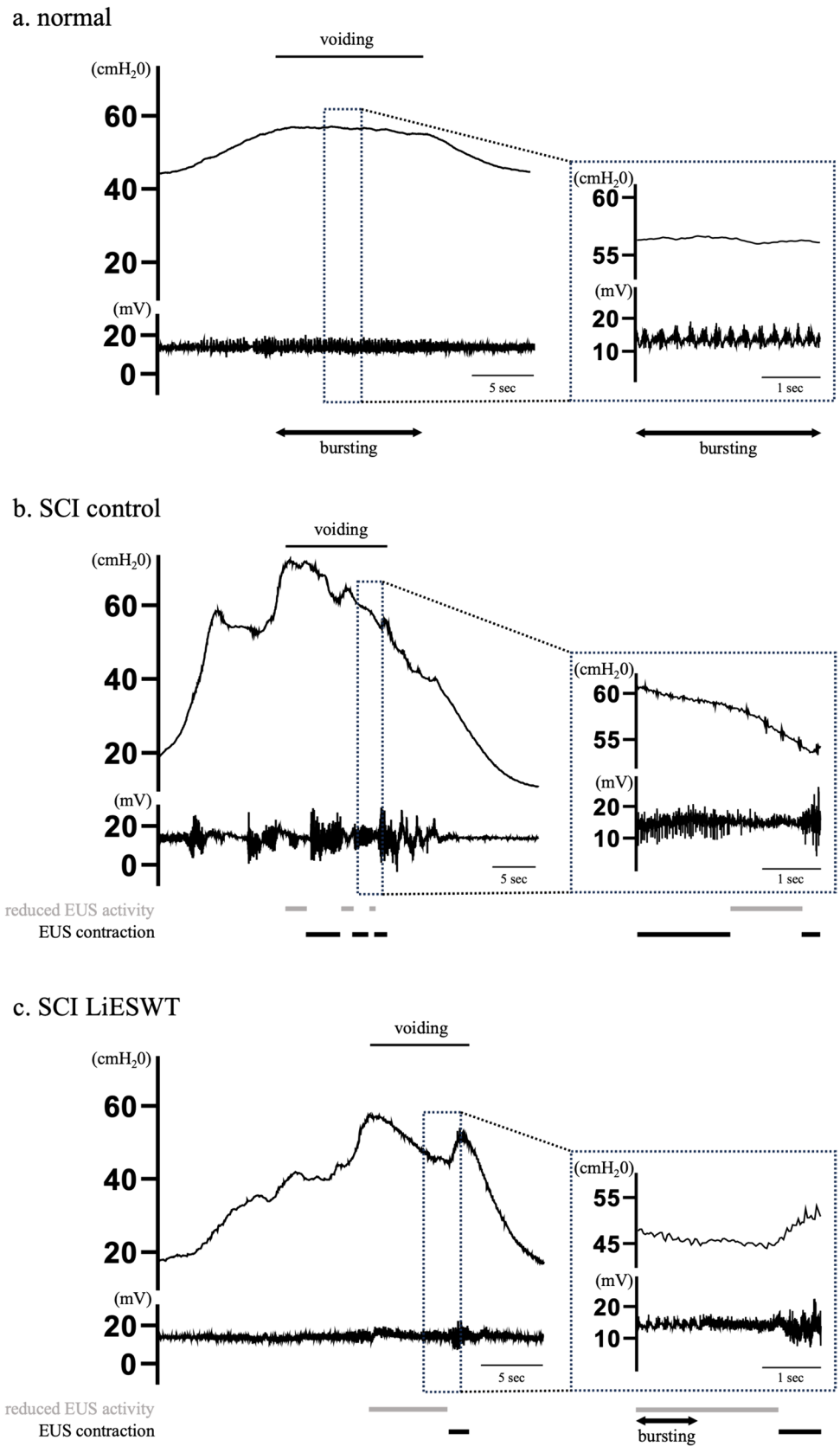

Fig. 4 Representative traces of simultaneous recording of cystometrogram and EUS-EMG during voiding in normal rats (**a**) and SCI rats treated without LiESWT (**b**) or with LiESWT (**c**). **a** EUS-EMG showed bursting activity during voiding. **b** EUS-EMG showed long EUS contraction duration and short reduced EUS activity during voiding. **c** EUS-EMG showed shorter EUS contraction duration and longer reduced EUS activity with bursting activity during voiding. *SCI* spinal cord injury, *LiESWT* low-intensity extracorporeal shock wave therapy

Table 2 Urethral parameters in EUS-EMG in the normal, SCI control, and SCI LiESWT groups

	Normal (n = 4)	SCI control (n = 8)	SCI LiESWT (n = 9)
EUS contraction duration with voiding (s)	–	5.17 ± 0.78	2.47 ± 0.50*
Duration of reduced EUS activity during voiding (s)	–	4.32 ± 0.59	5.05 ± 0.59
Reduced EUS activity ratio during voiding (%)	–	46.3 ± 5.1	66.9 ± 5.1*
Bursting activity ratio during voiding (%)	97.4 ± 1.8	4.0 ± 3.0†	10.6 ± 7.5†

Values are presented as mean ± standard error (SE)

SCI spinal cord injury, *LiESWT* low-intensity extracorporeal shock wave therapy, *EUS-EMG* external urethral sphincter electromyography

*$p < 0.05$ versus the SCI control group

†$p < 0.001$ versus the normal group

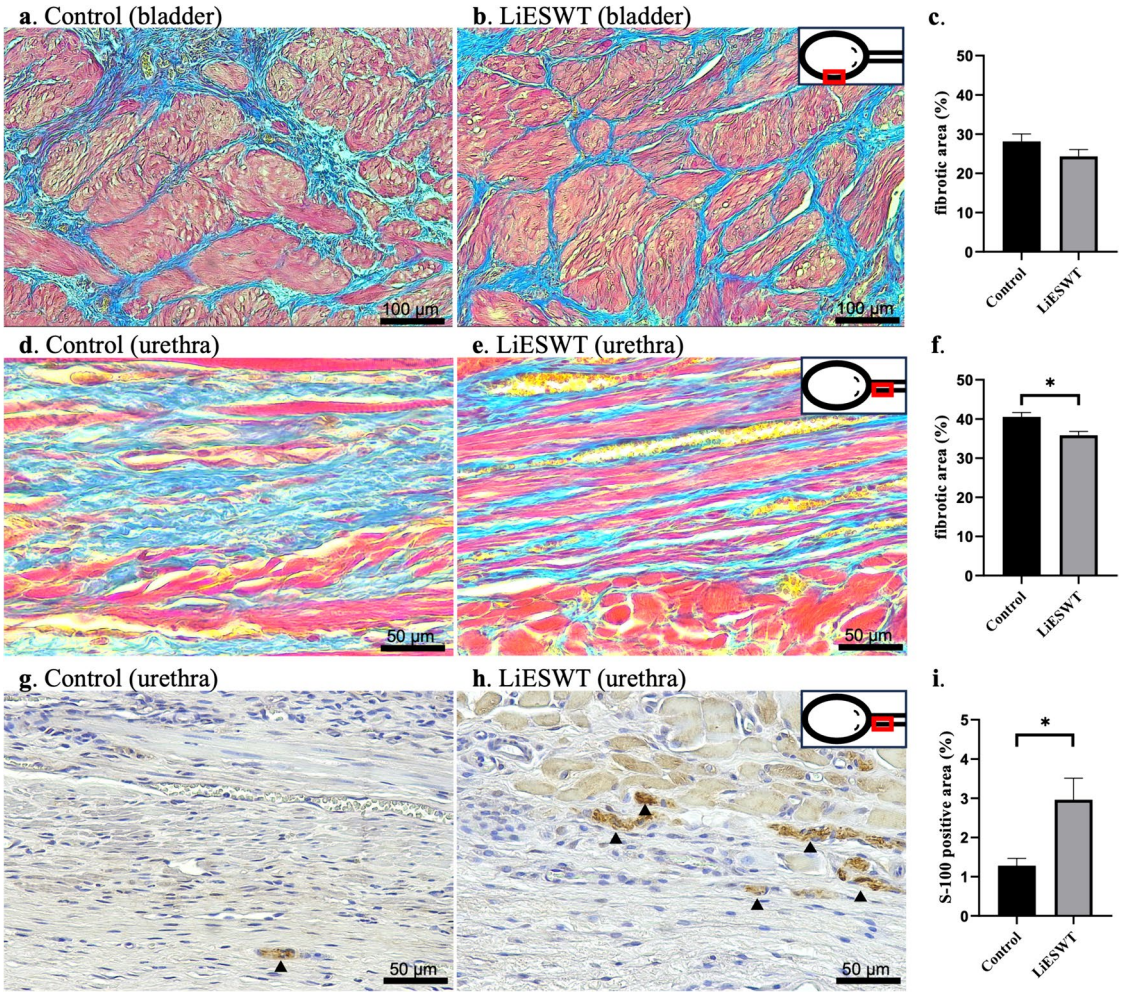

Fig. 5 **a**, **b**, **c** In Masson's trichrome staining of the bladder in the SCI control group (**a**) and the SCI LiESWT group (**b**), the percentage of the fibrotic area of the bladder (**c**) was not different. **d**, **e**, **f** In Masson's trichrome staining of the urethra in the SCI control group (**d**) and SCI LiESWT group (**e**), the percentage of the fibrotic area of the urethra (**f**) was significantly decreased in the SCI LiESWT group. **g**, **h**, **i** S-100 immunostaining of the urethra in the SCI control group (**g**) and the SCI LiESWT group (**h**), and the black arrows (▲) indicate positive S-100 staining of intermuscular nerves. The percentage of the S-100 positive area of the urethra (**i**) showed that the positive area was significantly increased in the SCI LiESWT group. Data are expressed as the mean ± standard error. *$p < 0.05$. *LiESWT* low-intensity extracorporeal shock wave therapy

bladder, but a significant decrease in the urethra. The bladder is overdistended after SCI and the LiESWT partially affected the bladder but had a greater effect on the urethra, possibly due to the ingenuity of an effective range of LiESWT, as shown in Fig. 1. Moreover, S-100 staining, an immunohistological marker of Schwann cells, was significantly increased in the urethra of the LiESWT group, suggesting that it may have promoted neural regeneration in the urethra. LiESWT is also expected to restore the damaged nerve (i.e., normal Aδ afferents), evidenced by improvement in pain in a rat model of interstitial cystitis through repair of damaged nerves [16], C-fiber degeneration, and the restoration of nerve expression in the urethral smooth muscle of diabetic rats [14]. The pelvic nerve contains somatic fibers innervating the EUS and facilitates pumping activity [24]; myelinated parasympathetic, sympathetic, or somatic nerves may work complementarily, especially in pathological conditions, and LiESWT may restore the damaged nerve involving coordinated action of the bladder and urethra. In a previous study [4] and in our previous studies [5, 7], desensitization of C-fiber afferent pathways by subcutaneously administered capsaicin or intrathecal injection of inhibitory neurotransmitters such as glycine, GABA, or opioids improved DSD. Therefore, another possibility is that the local effect of LiESWT on anti-inflammation, tissue, or nerve regeneration promoted neuromodulation in the interneurons of the spinal reflex, such as during electrical stimulation [25] or reflex plasticity associated with changes in the properties of neuropeptides, neurotrophic factors, or chemical receptors of the bladder/urethra afferent neurons in SCI [3]. However, these predictions require further analysis. Overall, LiESWT targeting the pelvic floor may restore peripheral nerve function around the urethra and reduce fibrosis. Future studies are needed to evaluate the different and precise effects of LiESWT on bladder and urethral activity.

The treatment options for SCI-related urinary disturbances are limited [7–14]. Treatments typically target the central nervous system and are often invasive and complicated. LiESWT is expected to be effective for DSD after SCI by suppressing tissue fibrosis and having an effect on peripheral nerves. Furthermore, it is minimally invasive, convenient, and can be treated repeatedly. The action of EUS during urination differs between species and sexes, and EUS bursting occurs during voiding bladder contractions in rats and dogs but not in humans [5, 19, 20]. However, LiESWT shows potential as a new treatment option for urinary disturbances, as LiESWT targeting the pelvic floor may restore peripheral nerve function around the urethra.

The current study has some limitations that warrant further consideration. First, we did not evaluate the long-term voiding function after LiESWT. It is unclear whether the therapeutic effects of LiESWT on CMG and EUS-EMG persist over time. Second, we did not monitor the activities of the nerves innervating the EUS. Third, we measured EUS-EMG activity in a small number of SCI rats, and all rats were females. Third, our transabdominal approach might not affect the urethra directly through the best therapeutic effects compared with the perineal approach. Despite these limitations, the results of the present study indicate the efficacy of LiESWT in EUS and suggest that it may be a promising treatment option for urinary disturbances associated with SCI.

Conclusion

This study provides evidence that LiESWT targeting the urethra after SCI can promote EUS relaxation and consequently ameliorate DSD. LiESWT, which is more convenient and less invasive than other treatment methods, may be a promising approach for the treatment of urinary disturbances after SCI, as shown in DSD models.

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Author contributions The contributions of each author are as follows: (1) Conception, experimental design, and investigation: KK, TCK, NK, and MM. (2) Critical drafting and revision of the article for important intellectual content: KK, TCK, NK, KN, TK, and MM. (3) Final approval of the version to be published: KK, TCK, NK, KN, TK, and MM. All contributors who do not meet these criteria for authorship are listed in the Acknowledgements section.

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Data availability The data sets of the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interests The authors have no competing interests to declare that are relevant to the content of this article.

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