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Effects of low-intensity extracorporeal shock wave therapy on lipopolysaccharide cystitis in a rat model of interstitial cystitis/bladder pain syndrome

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Abstract

Purpose To investigate the effect of low-intensity extracorporeal shock wave therapy (LiESWT) on lipopolysaccharide (LPS)-induced cystitis in an animal model of interstitial cystitis/bladder pain syndrome (IC/BPS).

Methods Sprague–Dawley rats were divided into three groups: control, cystitis (LPS group, intravesical injection of LPS (1 mg) twice), and cystitis with LiESWT (LiESWT group). On the third and fourth days, LiESWT was administered (0.12 mJ/mm^2, 300 shots each time) on the lower abdomen toward the bladder. On the seventh day, the rats underwent pain assessment and a metabolic cage study. Subsequently, a continuous cystometrogram (CMG) was performed under urethane anaesthesia. Immunohistochemical studies were also performed, including S-100 staining, an immunohistochemical marker of Schwann cells in the bladder.

Results In the LPS group, the pain threshold in the lower abdomen was significantly lower than that in the control group. In the metabolic cage study, the mean voided volume in the LPS group significantly increased. The CMG also revealed a significant decrease in bladder contraction amplitude, compatible with detrusor underactivity in the LPS group. Immunohistochemical studies showed inflammatory changes in the submucosa, increased fibrosis, and decreased S-100 stain-positive areas in the muscle layer of the LPS group. In the LiESWT group, tactile allodynia and bladder function were ameliorated, and S-100 stain-positive areas were increased.

Conclusion By restoring nerve damage, LiESWT improved lower abdominal pain sensitivity and bladder function in an LPS-induced cystitis rat model. This study suggests that LiESWT may be a new therapeutic modality for IC/BPS.

Keywords Low-intensity extracorporeal shockwave therapy · Interstitial cystitis · Bladder pain syndrome · Detrusor underactivity · Nerve regeneration

Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic inflammatory disease affecting the bladder wall. IC/BPS symptoms are characterised by persistent pain in the lower abdomen and pelvis, accompanied by urinary frequency and urgency [1], thus disturbing the patient's quality of life (QOL) [2]. Furthermore, patients experiencing IC/BPS incur higher healthcare costs compared to those without the condition, thus posing a cost issue [3]. This disease is more prevalent in women than men; for instance, 2.7–6.5% of adult females in the US experience IC/BPS [4]. The subtypes are cystoscopically classified into Hunner and non-Hunner lesions [5]. However, a comprehensive

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understanding of the pathogenesis of this disease remains elusive, and no curative treatment exists.

Several IC/BPS animal models have been proposed [6]. Nonetheless, a standard model has yet to be established. Intraperitoneally or intravesically administered lipopolysaccharide (LPS) has been used to induce cystitis in animal models, which have been used as IC/BPS models because they mimic the pathogenesis of IC/BPS and sustain bladder inflammation over an extended period [6, 7]. In addition, pathohistological studies of these models revealed inflammatory cell infiltration and increased fibrosis in the bladder, resembling the histopathological features observed in patients with IC/BPS [6–8]. Intravesical injection of LPS also induces urinary disturbances and heightened sensitivity to lower abdomen pain [7, 9]. Therefore, the intravesical LPS-induced cystitis model may effectively replicate lower urinary tract dysfunction, such as IC/BPS, thereby serving as a valuable model for evaluating this disease.

Low-intensity extracorporeal shock wave therapy (LiESWT) is currently used clinically in orthopaedic, angina pectoris, and erectile dysfunction therapy with minimal adverse effects [10–12]. Preclinical studies have demonstrated its anti-inflammatory and angiogenic effects [13, 14]. Previous studies have indicated that LiESWT is effective in models of lower urinary tract symptoms. In a rat model of capsaicin-induced prostatitis, LiESWT suppressed cytokine-positive cells in the prostate and improved scrotal pain sensitivity [15]. Furthermore, LiESWT facilitated bladder nerve regeneration and improved bladder contractility in a diabetic bladder dysfunction rat model [16]. Thus, we hypothesised that LiESWT could effectively treat IC/BPS, characterised by multifocal unknown pathogeneses such as inflammation, fibrosis, and nerve damage.

Therefore, in the present study, we investigated voiding and pain behaviour, bladder function, and bladder morphology and immunostaining for a marker of Schwann cells, S-100 stain [17], to explore the therapeutic effect and mechanism of LiESWT in an LPS-induced IC/BPS model in rats.

Materials and methods

Animals

A total of 24 adult female Sprague–Dawley rats (204–307 g) (Kyudo, Saga, Japan) were used. These rats were housed under 12 h artificial light/dark cycles with free access to food and water. The animals were randomly divided into three groups: a control group (n = 8), a cystitis group exposed to LPS (LPS group) (n = 8), and a cystitis group treated with LiESWT (LiESWT group) (n = 8).

Study protocols

The study protocol is presented in Fig. 1. In the LPS and LiESWT groups, LPS was injected into the bladder on days zero and one, respectively. On days three and four, LiESWT was administered to the LiESWT group. On day six, all rats underwent the von Frey test and metabolic cage study, and the next day (day 7), they underwent a continuous cystometrogram (CMG) and histological study.

Induction of LPS-induced cystitis

The rats were anaesthetised with an intraperitoneal injection of ketamine hydrochloride (90 mg/kg; Daiichi Sankyo Propharma Co. Ltd., Tokyo, Japan) and xylazine hydrochloride (10 mg/kg; Elanco Japan Inc., Tokyo, Japan). A polyethylene catheter (BD Intramedic™ Polyethene Tubing PE-50; Becton, Dickinson and Company, NJ, USA) was inserted into the bladder via the urethra. First, the bladder was emptied, followed by intravesical infusion of 0.2 ml of LPS from Escherichia coli O55:B5 (Sigma-Aldrich Co., LLC, MO, USA) at a concentration of 5 mg/mL in sterilised saline. The LPS solution was allowed to remain in the bladder for 60 min. After LPS exposure, the bladder was allowed to drain freely through the open catheter end. This

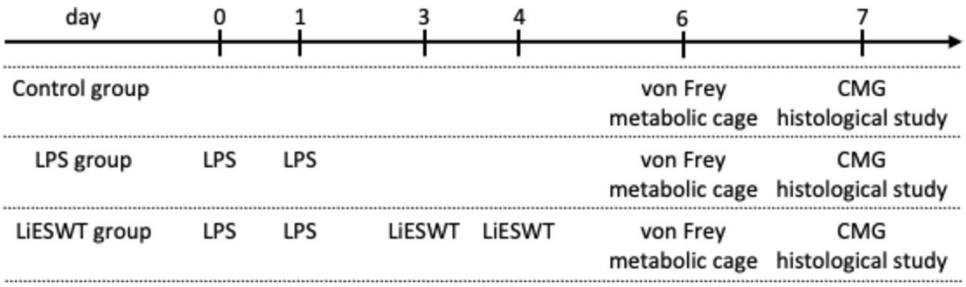

Fig. 1 Study protocol. In the LPS and LiESWT groups, LPS was injected into the bladder on days zero and one, respectively. LiESWT was administered to rats in the LiESWT group on days three and four. On day six, all rats underwent the von Frey test and metabolic cage study, and the next day (day seven), they underwent a continuous cystometrogram (CMG) and histological study

treatment was repeated every 24 h for two days to induce cystitis.

LiESWT procedure

The shock waves were produced using an ED-1000 (Medispec, Yehud, Israel). The rats were anaesthetised with isoflurane and placed in a supine position, and their lower abdomen was shaved. An ultrasound gel pad (Yasojima Proceed Co. Ltd., Hyogo, Japan) was applied between the probe and the skin of the lower abdomen to ensure optimal coupling (Fig. 2). The probe was specifically designed to focus on the rat bladder. Subsequently, LiESWT was applied to the pelvis targeting the bladder with an energy flux of 0.12 mJ/mm^2 at a frequency of 2 Hz, and 300 shots were modified from previous reports [18, 19].

Tactile allodynia assessment

The von Frey test was used to assess tactile sensitivity. Rats were placed in a stimulation chamber with a wire mesh bottom and left for 1 h to acclimatise to the environment. Von Frey monofilaments (Stoelting Co., Wood Dale, IL, USA) were applied to the lower abdomen close to the urinary bladder and hind paw. Positive responses to stimulation encompassed the following: (1) jumping, (2) rapid retraction from stimulation, and (3) licking of the stimulated area. The withdrawal threshold was calculated using the up-down method for 20 trials. The 50% withdrawal threshold was formulated from the mean of the logarithmic tabular values [$\log_{10}$ (force in g $\times 10^4$)] for the patterns of positive and negative responses [20].

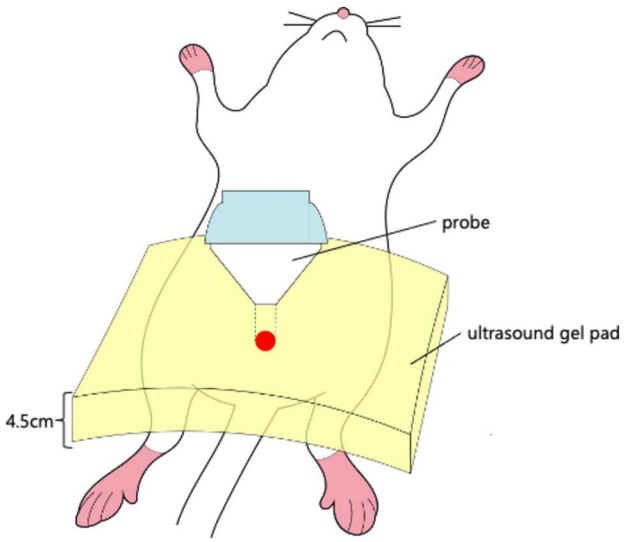

Fig. 2 LiESWT procedure. The shock waves were produced using an ED-1000. The rats were anesthetized with isoflurane and placed supine with their lower abdomen shaved. The ED-1000 has a therapeutic zone of 9 mm in diameter and 130 mm in depth from the apex of the probe, of which up to a depth of 60 mm from the contact area, the therapeutic effect is weakened by interference between shock waves. In this study, to prevent weakening therapeutic effect, an echo gel pad 45 mm thick was applied between the skin and probe, and the probe was applied at an angle toward the inside of the pelvis, assuming a rat bladder at a depth of about 60–90 mm from the apex of the probe. LiESWT was applied at an energy flux of 0.12 mJ/mm^2 at 2 Hz for 300 shots per treatment

Metabolic cage study

After the von Frey test, each rat was placed in a metabolic cage (Metabolica™, Sugiyama-gen Co. Ltd., Tokyo, Japan) while awake and allowed unrestricted movement for 24 h. Urinary volume was continuously measured with PowerLab (ADInstruments Pty. Ltd., New South Wales, Australia). All rats had free access to water and food during the recording. The mean voided volume was calculated by dividing the total urinary volume (g) by the urinary frequency per 24 h.

Continuous cystometrogram (CMG)

The rats were anaesthetised with isoflurane, and catheter implantation was performed. The right jugular vein was exposed, and a polyethylene catheter (BD Intramedic™ Polyethene Tubing PE-10, Becton, Dickinson and Company, NJ, USA) was inserted into the vessel for drug injection. The bladder and ureters were exposed through a lower midline abdominal incision. The bilateral ureters were cut, and the distal ends were tied. A PE-50 polyethylene catheter with a cuff was inserted into the bladder through the bladder dome to record the intravesical pressure. The abdomen was closed with sutures. Subsequently, isoflurane was turned off and replaced by one-third intraperitoneal urethane and the remaining two-thirds subcutaneous urethane, a total of 1 g/kg (Sigma-Aldrich Co., LLC, MO, USA). The intravesical catheter was connected via a three-way stopcock to a pressure transducer to record intravesical pressure, and a syringe pump was infused with saline. The bladder was infused with physiological saline at a rate of 0.05 ml/min during the cystometry. PowerLab (ADInstruments Pty. Ltd., New South Wales, Australia) was used for data amplification, acquisition, and manipulation. The intercontraction interval (ICI), pressure threshold (PT), baseline pressure, amplitude of voiding pressure, and number of non-voiding contractions (NVCs) were measured when rhythmic bladder contractions stabilised for at least 30 min. After the final contraction, bladder infusion was stopped, and the residual volume (RV) was measured by withdrawing intravesical fluid through the catheter by gravity. NVCs were defined as contractions of >7 cmH_2O from the baseline pressure that occurred during the filling phase. After CMG, the animals were sacrificed, and their bladders were harvested and weighed. Bladder weight divided by body weight was compared among the three groups.

Histology and immunohistochemistry

Each harvested bladder was fixed in buffered 10% formaldehyde, paraffin-embedded, and cut into 4-µm-thick sections. The sections were subjected to haematoxylin and eosin (H&E), Masson's trichrome, and S-100 staining. All the slides were dewaxed in xylene, dehydrated in graded alcohol, and rehydrated in distilled water. H&E (HAE-1-1FU, ScyTek Laboratories Inc., UT, USA) and Masson's trichrome (TRM-1-1FU, ScyTek Laboratories Inc. Lcc, UT, USA) were performed using the respective kits, according to the manufacturer's instructions. Three randomly selected high-power fields (objective × 20) surrounding the muscle layer were analysed to quantify the fibrotic area to eliminate potential submucosal collagenous tissue. 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 immunohistochemical examination, deparaffinised sections were first treated with 3% H_2O_2 for 10 min to remove endogenous peroxidase activity and then incubated with 2.5% normal horse serum for 2 h at room temperature to block nonspecific background. The sections were then incubated with primary rabbit antibodies against S-100 (1:2000, GTX48819, GeneTex, Irvine, CA, USA) at 4 °C overnight. Subsequently, the sections were incubated with a horse anti-rabbit antibody for 30 min. Finally, the reaction sites were stained with diaminobenzidine (DAB) chromogen until the desired stain intensity was developed, and the sections were then counterstained with haematoxylin. A negative control was performed to elucidate nonspecific immunostaining without the primary antibody. Three randomly selected fields (objective × 10) were analysed to quantify peripheral nerves in the bladder. The area of the S-100 stain-positive range in each section was calculated using FIJI ImageJ software.

Statical analysis

Data are presented as mean ± standard error (SE). A one-way analysis of variance followed by the Tukey–Kramer test for post hoc comparisons was performed for intergroup comparisons. Statistical significance was set at $p < 0.05$.

Results

Body weight and bladder weight among the three groups

In the LPS group (n = 8), bladder weight to body weight significantly increased up to 1.13 ± 0.26 mg/g compared to the control group (n = 8) (Table 1, 0.44 ± 0.02 mg/g, $p < 0.05$), while it recovered in LiESWT group (n = 8) (Table 1, 0.79 ± 0.05 mg/g).

Table 1 Comparison of body and bladder weights among three groups

	Control	LPS	LiESWT
Body weight (g)	221.1 ± 3.9	257.4 ± 13.0	247.0 ± 11.6
Bladder weight (mg)	99.2 ± 3.4	285.4 ± 60.8*	203.3 ± 17.2
Weight ratio (bladder weight/body weight)	0.44 ± 0.02	1.13 ± 0.26*	0.79 ± 0.05

Data are expressed as the mean ± standard error

*$p < 0.05$ vs. Control group

Differences in pain threshold among the three groups

In the LPS group (n = 8), the pain threshold on the lower abdomen was significantly lower than that in the control group (n = 8) (Fig. 3A, 3.97 ± 0.43 vs. 5.62 ± 0.32; $p < 0.01$). In contrast, the pain threshold in the LiESWT group (n = 8) significantly higher than that in the LPS group (n = 8) (Fig. 3A, 5.15 ± 0.16 vs. 3.97 ± 0.43; $p < 0.05$). The pain threshold on the hind paw was not significantly different among the three groups (Fig. 3B, 5.58 ± 0.21 vs. 4.92 ± 0.21 vs. 5.32 ± 0.17).

Differences in mean voided volume and urinary frequency among the three groups

In the LPS group (n = 8), the mean voided volume in the light and dark phases was significantly higher than that in the control group (n = 8) (Table 2, in the light phase: 1.86 ± 0.41 ml vs. 0.89 ± 0.07 ml, $p < 0.05$; in the dark phase: 1.58 ± 0.34 ml vs. 0.70 ± 0.04 ml, $p < 0.05$). However, in the LiESWT group (n = 8), it recovered to the level in the control group (Table 2, in the light phase: 1.07 ± 0.09 ml vs. 0.89 ± 0.07 ml, $p = 0.86$; in the dark phase: 0.80 ± 0.10 ml vs. 0.70 ± 0.04 ml, $p = 0.94$). There were no differences in urinary frequency in 24 h among the three groups (Table 2, 30.4 ± 1.6 vs. 28.6 ± 2.8 vs. 33.3 ± 1.6); however, that in the dark phase was significantly higher in the LiESWT group than in the LPS group (Table 2, 24.8 ± 2.0 vs. 17.9 ± 1.8, $p < 0.05$).

Differences in bladder activity

In the LPS group (n = 8), the contraction amplitude was significantly lower than that in the control group (n = 8) (Table 3, 13.95 ± 2.09 cmH_2O vs. 19.51 ± 0.82 cmH_2O; $p < 0.05$) as presented in Fig. 4. The ICI in the LPS group

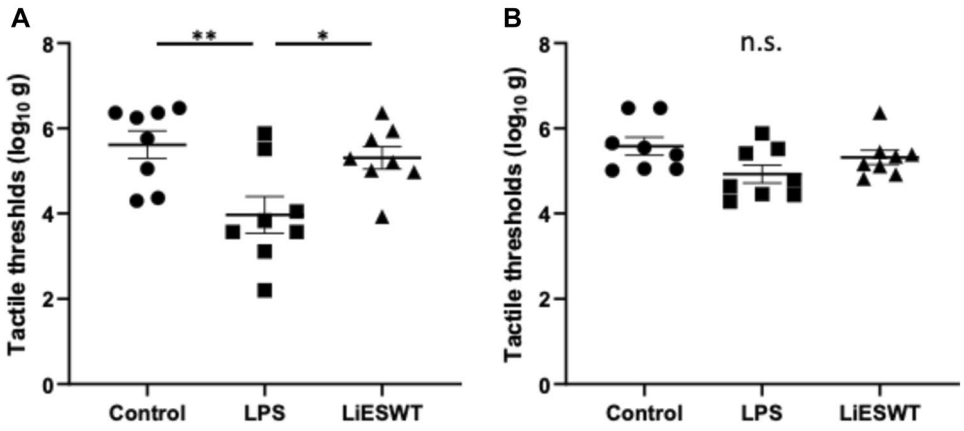

Fig. 3 von Frey test. The logarithm thresholds for withdrawal responses to filament stimulation on the (**A**) lower abdomen and (**B**) hind paws. **A** Pain threshold in the lower abdomen was significantly lower in the LPS group than in the control group. However, it was restored in the LiESWT group. **B** Pain threshold in the hind paw was not significantly different among the three groups. Data are expressed as mean ± standard error, and individual data are dot-plotted. *p < 0.05, **p < 0.01, *n.s.* not significant

Table 2 Comparison of bladder functions in metabolic cage study among three groups

	Control	LPS	LiESWT
24 h urine production (g)	22.83 ± 1.45	39.73 ± 4.96*	28.67 ± 3.98
Voided volume (g)			
Light phase	0.89 ± 0.07	1.86 ± 0.41*	1.07 ± 0.09
Dark phase	0.70 ± 0.04	1.58 ± 0.34*	0.80 ± 0.10†
24-h	0.76 ± 0.04	1.60 ± 0.36*	0.85 ± 0.09
Urinary frequency (times)			
Light phase	10.3 ± 0.8	8.6 ± 1.3	8.5 ± 1.0
Dark phase	19.9 ± 1.2	17.9 ± 1.8	24.8 ± 2.0†
24-h	30.4 ± 1.6	28.6 ± 2.8	33.3 ± 1.6

Data are expressed as the mean ± standard error

*p < 0.05 vs. Control group, †p < 0.05 vs. LPS group

had a tendency to be prolonged compared to that in the control group (Table 3, 668.57 ± 97.48 s vs. 435.53 ± 44.05 s; p = 0.0851); however, this was not statistically significant. In the LiESWT group (n = 8), the contraction amplitude was significantly higher than that in the LPS group (Table 3, 19.23 ± 1.21 cmH_2O vs. 13.95 ± 2.09 cmH_2O; p < 0.05) and returned to the control level. The residual urine volume was not significantly different among the three groups (Table 3. 0.11 ± 0.04 ml vs. 0.68 ± 0.52 ml vs. 0.12 ± 0.06 ml). However, in the LPS group, one rat (12.5%) experienced urinary retention.

Histological and immunohistological response

Haematoxylin and eosin staining revealed submucosal inflammatory changes in the LPS group (n = 8). However, these changes were ameliorated in the LiESWT-treated group (n = 8). Masson's trichrome staining showed that the percentage of the fibrotic area in the LPS group (n = 8) was significantly higher than that in the control group (n = 8) (Fig. 5G, 17.65 ± 0.37% vs. 10.60 ± 1.08%; p < 0.01). In the LiESWT group (n = 8), the extent of this area was decreased, and the percentage was significantly decreased compared to that in the LPS group (n = 8) (Fig. 5G, 13.90 ± 1.01% vs. 17.65 ± 0.37%; p < 0.05). In S-100 staining, the positive area was significantly decreased in the LPS group (n = 8) compared with that in the control group (Fig. 5K,

Table 3 Comparison of bladder functions in cystometry among three groups

	Control	LPS	LiESWT
Base line pressure (cmH_2O)	5.27 ± 0.54	7.83 ± 1.11	5.17 ± 0.57
Threshold pressure (cmH_2O)	8.54 ± 0.58	10.84 ± 1.60	8.76 ± 1.11
Amplitude of bladder contraction (cmH_2O)	19.51 ± 0.82	13.95 ± 2.09*	19.23 ± 1.21†
Intercontraction intervals (s)	435.53 ± 44.05	668.57 ± 97.48	475.17 ± 71.41
Non-voiding contractions (times)	0.00 ± 0.00	1.29 ± 0.75	1.13 ± 0.79
Residual urine volume (ml)	0.11 ± 0.04	0.68 ± 0.52	0.12 ± 0.06

Data are expressed as the mean ± standard error

*p < 0.05 vs. Control group, †p < 0.05 vs. LPS group

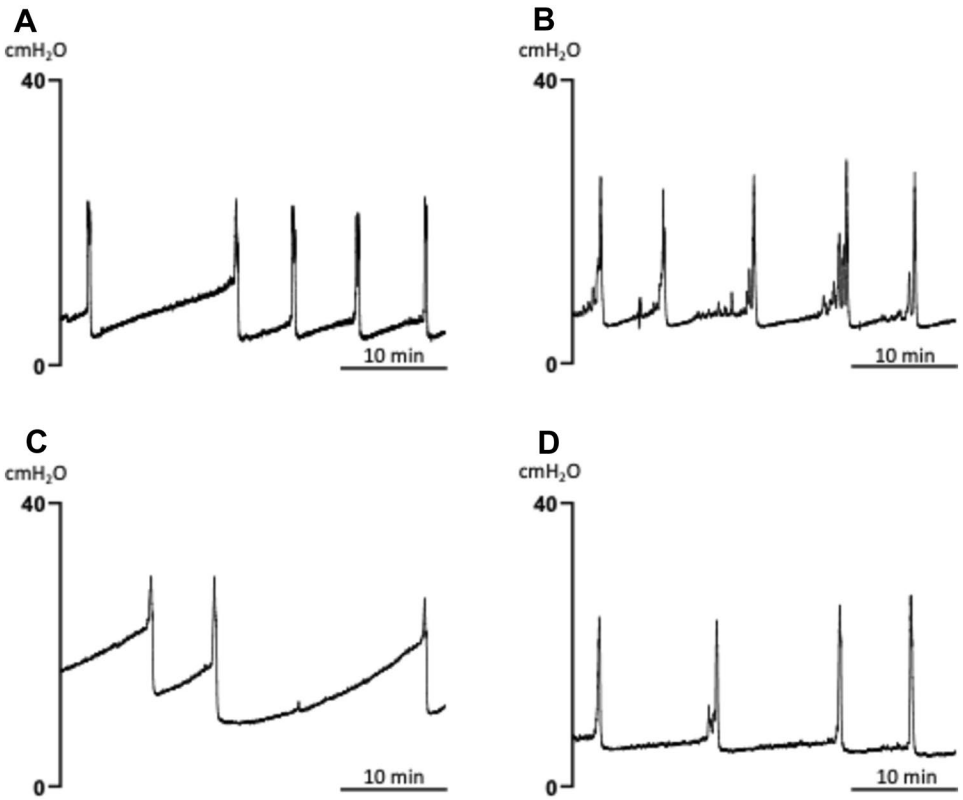

Fig. 4 Representative traces of continuous cystometrogram. The control group exhibited a regular and stable pattern (**A**). Two patterns of bladder activity were observed in the LPS group: unstable voiding functions characterized by non-voiding contractions (**B**); and prolonged micturition intervals with low voiding amplitude (**C**). The LiESWT group showed improved voiding function characterized by regular micturition intervals and restored voiding amplitude (**D**). The scale bar indicates 10 min

3124 ± 728 μm^2 vs. 5737 ± 1040 μm^2; $p < 0.05$). In the LiESWT group ($n = 8$), the positive area was significantly increased compared with that in the LPS group ($n = 8$) (Fig. 5K, 6176 ± 1140 μm^2 vs. 3124 ± 728 μm^2; $p < 0.05$).

Discussion

The results of this study have several important implications. First, the LPS-induced cystitis model mimics multifactorial IC/BPS morphology, as evidenced by the histological changes in the infiltration of inflammatory cells in the urothelium and an increase in fibrosis. Second, the LPS-induced cystitis model mimics the chronic model of detrusor underactivity, as evidenced by an increase in the voided volume in the metabolic cage study and decreased bladder contractility in the CMG. Third, LiESWT improves this unique model with pain and bladder dysfunction, as evidenced by an amelioration of the pain threshold on the lower abdomen, a decrease in the voided volume in the metabolic cage study, and an increase in bladder contractility in CMG. Fourth, LiESWT has a distinct mechanism to improve the LPS-induced model, as evidenced by the inhibition of fibrosis and restoration of the decreased Schwann cell marker S-100.

In this study, we used an intravesical injection of LPS to induce the disease model. LPS is a major component of the outer membrane of gram-negative bacteria that triggers innate immune responses in the human body [21]. LPS injected into the bladder has been reported to lower the pain threshold in the lower abdomen in the von Frey test, shorten the voiding interval in CMG, and decrease the voided volume, similar to interstitial cystitis [7, 9]. In addition, increased inflammatory cell infiltration and fibrosis have been reported [7, 9]. In the present study, intravesical injection of LPS induced pain sensitivity in the lower abdomen and histologically increased inflammatory cell infiltration and fibrosis. These results are similar to those of previous studies [7, 9]. On the other hand, unlike previous studies [7, 9], in continuous CMG and metabolic cage studies, the ICI had a tendency to be prolonged, and the voided volume was increased. In addition, in the present study, bladder contractility was decreased, and one out of eight rats in the LPS group developed urinary retention. Histologically, S-100 staining, an immunohistochemical marker of Schwann cells, in the muscle layer decreased in the LPS group. These results suggest that myelinated nerves responsible for normal bladder sensation/output, such as Aδ fibers, were strongly injured in this study, which may have led to different results for large bladder capacity with decreased bladder contractility. We have also reported that nitric oxide-mediated urethral smooth muscle relaxation during voiding is important for efficient micturition and bladder contraction [22, 23]. Another possibility is that our LPS model might have

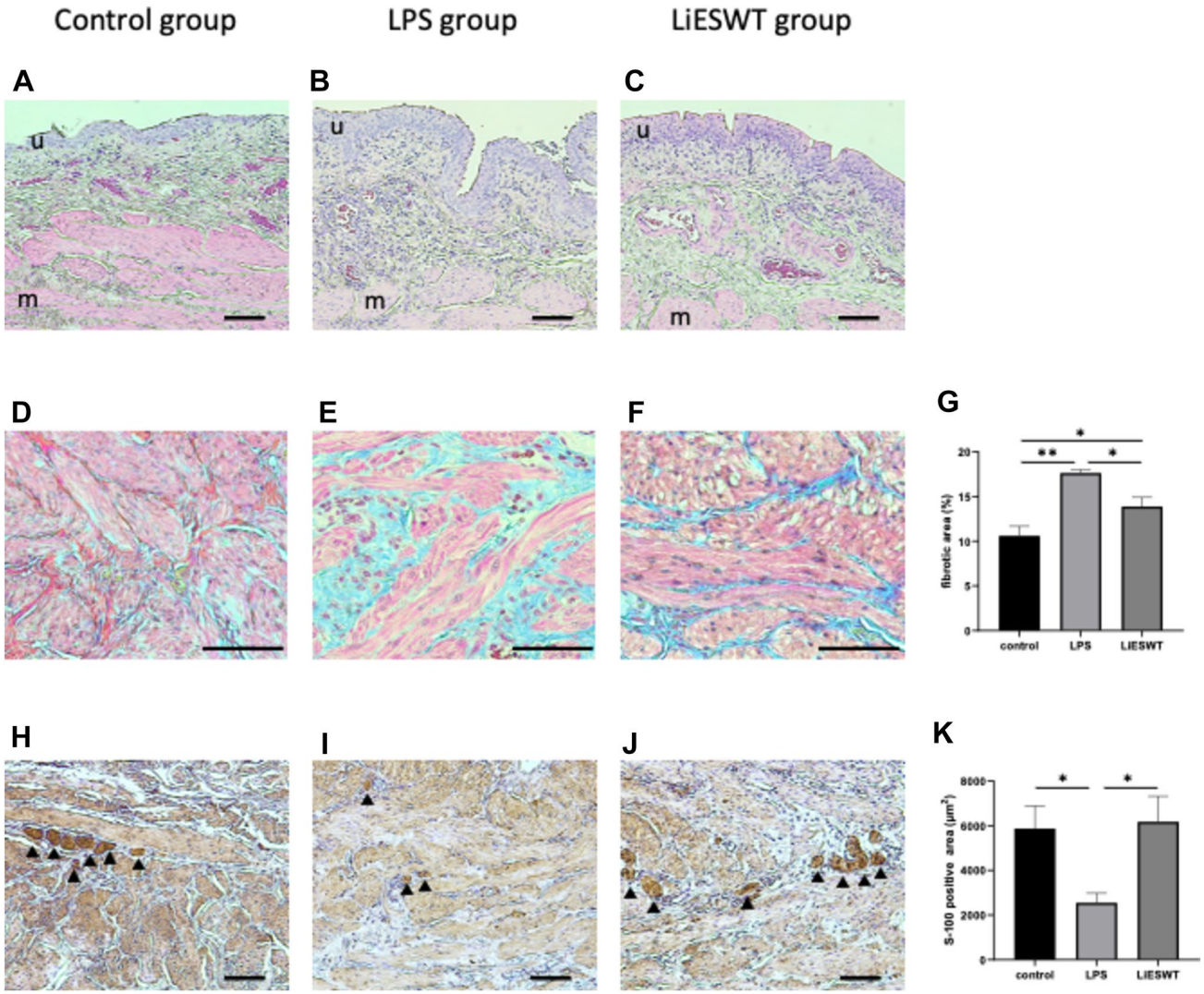

Fig. 5 Histology and Immunohistochemistry. **A**–**C** Illustrating the microscopic finding (objective × 10) of H&E stain. *u* urothelium, *m* muscle. **D**–**F** Illustrating the microscopic finding (objective × 20) of Masson's trichrome stain. All pictures show muscle layers. **G** The percentage of the fibrotic area. **H**–**J** Illustrating the microscopic finding (objective × 10) of S-100 stain. Black arrows (▲) indicate positive S-100 staining of intermuscular nerves. **K** The area of the S-100 stain-positive range. All scale bars represent 100 μm. Data are expressed as the mean ± standard error. *p < 0.05, **p < 0.01. Haematoxylin and eosin (H&E) staining showed inflammatory changes in the submucosa (**B**), which were ameliorated in the LiESWT group (**C**). Masson's trichrome staining showed that the area of fibrosis was significantly increased in the LPS group (**E**) compared to that in the control group (**D**), while it was significantly decreased in the LiESWT group (**F**). S-100 staining showed that positive area was significantly decreased in the LPS group (**I**) compared to that in the control group (**H**), while the area was significantly increased in the LiESWT group (**J**)

affected bladder and urethral function. Another possibility is the differences in the number of LPS injections and the period between LPS injections and experiments when compared with previous studies [7, 9]. Overall, lower abdominal pain and bladder dysfunction recovered after LiESWT, suggesting that this model was appropriate for examining the effects of LiESWT.

Extracorporeal shock wave therapy (ESWT) was first used to treat kidney stones in the 1980s [24] and remains a standard treatment for urinary stones. LiESWT, which is at lower energy levels than that used in lithotripsy, has proven that when shock waves hit tissue, they induce cavitation that might generate a physical force as "shear stress" on the cell membrane and ultimately exert biological effects [13]. In the present study, LiESWT significantly increased the pain threshold in the lower abdomen, improved bladder hypertrophy with fibrosis, and decreased inflammatory cell infiltration into the bladder. We also found that the bladder contraction amplitude was increased by 38% in the CMG, and the mean voided volume was decreased by 47% in the metabolic cage study in the LiESWT group compared with that in the LPS group. Bladder contractility is very

important for efficient urination; a decrease in bladder contractility leads to a low flow of urination and increased residual urine volume [25, 26]. Chuang et al. reported that LiESWT increased Ki-67 positive cells and restored bladder contractility in a cryoinjury-induced myogenic detrusor underactivity rat model, suggesting that LiESWT regenerates muscle and improves voiding function [27]. In diabetic bladder dysfunction model rats, LiESWT has also been reported to improve bladder contractility by activating angiogenesis and bladder innervation [16, 28]. These findings suggest that LiESWT can restore the chronic bladder dysfunction model through muscle restoration and angiogenesis. In addition, LiESWT increased S-100 staining-positive area in the bladder wall in the present study. LiESWT has been reported to significantly increase neuronal nitric oxide synthase (nNOS) in urethral smooth muscle and restore urethral function in diabetic bladder dysfunction model rats [28]. Furthermore, LiESWT significantly elevated the number of nNOS-positive cells in the penis of erectile dysfunction model rats and improved its function [29]. Wang et al. reported a significant upregulation of brain-derived neurotrophic factor (BDNF) in erectile dysfunction model rats with cavernous nerve injury, achieved via activation of the PERK/AF4 pathway in vitro [30]. Considering the presumed relation of nNOS and BDNF to nerve regeneration [31, 32], the increase in the S-100 positive area in the bladder may have been influenced by nNOS and BDNF synthesis. Overall, this is the first report to describe the mechanisms of peripheral nerve regeneration in an IC/BPS animal model. Further studies are necessary to clarify this point.

The primary symptom of IC/BPS is chronic lower abdominal and pelvic pain, a serious problem that reduces a patient's QOL [2]. In preclinical studies, LiESWT decreased inflammatory molecules such as cyclooxygenase-2 (COX-2) and tumour necrosis factor-α (TNF-α) in the prostate and ameliorated tactile allodynia in capsaicin-induced prostatitis model rats [15]. In addition, combination therapy with melatonin and LiESWT in a rat model of neuropathic pain with chronic constriction injury to the sciatic nerve reduced the expression of inflammatory proteins in dorsal ganglia neurons. It increased the pain threshold of the hind paw compared to that in rats treated with melatonin alone [33]. In the present study, LiESWT significantly increased the pain threshold only in the lower abdomen, indicating bladder innervation and histologically decreased inflammatory cell infiltration into the bladder, which is similar to previous studies [15, 33]. Based on previous and present findings, LiESWT could improve pain through anti-inflammatory effects and restoration of damaged nerves.

The effects of LiESWT on other animal models of IC/BPS have been reported. In a study by Wang et al. involving an HCl-induced cystitis model rat, it was noted that LiESWT may reduce bladder inflammation by attenuating the mitochondrial-dependent apoptotic pathway, resulting in improved urinary frequency [34]. Similarly, in a study investigating autoimmune cystitis induced by uroplakin 3A, LiESWT was also reported to reduce inflammatory cells and inflammatory markers such as TNF-α and improve lower abdominal pain and bladder function [35]. This study also showed that LiESWT effectively relieves inflammation and improves pain and bladder function with a neuro-regenerative effect.

The clinical benefits of LiESWT have been demonstrated. Kikuchi et al. reported that LiESWT significantly improved chest pain symptoms and cardiac function in patients with severe angina pectoris without complications compared to the sham group [11]. Furthermore, Kitrey et al. indicated that in severe erectile dysfunction patients who were phosphodiesterase type 5 inhibitor non-responders, LiESWT significantly improved erectile rigidity without complications [12]. LiESWT may be helpful in many other diseases and clinical settings. In particular, the pathogenesis of IC/BPS is not yet fully understood. Although stress relief, oral medications, dimethyl sulfoxide intravesical instillation, and hydrodistention with coagulation have been used in clinical practice [36], their effects are not curative, have a short duration, and are invasive. Chuang et al. investigated the effect of LiESWT on patients with IC/BPS in a clinical setting. They reported that LiESWT did not improve O'Leary-Sant symptom scores or urinary frequency compared to a placebo, but a significantly larger number of patients recovered a visual analogue scale of 3 or more without any adverse events [37], which suggests that LiESWT can affect pain reduction in IC/BPS patients. In the future, modifying the energy flux and frequency of LiESWT may further enhance urinary function, making this treatment a potential candidate for minimally invasive treatment in patients with IC/BPS.

The present study has some limitations. First, LiESWT was performed on days three and four after the first intravesical investigation of LPS, when cystitis may not have been established. Therefore, this study indicates a preventive rather than a therapeutic effect of LiESWT. Second, we used only female rats, possibly due to sex differences in the model and the effects of LiESWT. However, IC/BPS is more prevalent in women than men [4], so this study may have the advantage of examining the effect of LiESWT on IC/BPS. Third, the LiESWT protocol used in this study may not have had an optimal shock wave dosage and frequency. Fourth, the LPS cystitis model employed deviated from the typical IC/BPS model characterised by urinary frequency; rather, it exhibited a larger bladder capacity with decreased bladder contractility. Despite these limitations, the results of the present study may be beneficial for future studies on the effects of LiESWT on IC/BPS.

Conclusions

We demonstrated that LiESWT significantly alleviated lower abdominal pain and improved bladder function in an LPS-induced cystitis rat model. LiESWT may exert this effect by reducing inflammation and restoring peripheral nerve damage.

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Availability of data and material The data sets of the current study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval The study protocol was approved by the University of Ryukyu Institutional Animal Care and Use Committee (protocol no. A2020077, A2020019) in compliance with the ARRIVE guidelines.

Consent for publication Not applicable.

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