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Long-term functional change of cryoinjury-induced detrusor underactivity and effects of extracorporeal shock wave therapy in a rat model

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Abstract

Purpose To investigate the long-term functional change of cryoinjury-induced detrusor underactivity (DU) and the therapeutic potential of repeated low-energy shock wave therapy (LESW).

Methods Fifty-six female Sprague–Dawley rats were assigned into sham and cryoinjury of bladder with or without LESW (0.05 or 0.12 mJ/mm^2; 200 pulses; twice a week for 2 weeks after cryoinjury). Under halothane anesthesia, an incision was made in lower abdomen, and cryoinjury was provoked by bilateral placement of a chilled aluminum rod on the bladder filled with 1 ml saline. Measurement of contractile responses to KCl and carbachol in vitro, conscious voiding, and histological and protein changes were performed on week 1, 2, and 4 after cryoinjury.

Results Cryoinjury of bladder induced a significant decrease in the detrusor contraction amplitude at week 1 (55.0%) and week 2 (57.2%), but the decrease in the contractile response to KCl and carbachol was only noted at week 1. At week 1, significantly increased COX-2 and TGF-β1 expression accompanied a decrease of VEGF and CGRP expression. At week 4, there was a partial recovery of voiding function and a significant increase in the Ki-67 staining. LESW treatment at higher energy level further amplified the Ki-67 staining and improved the recovery of contraction amplitude and the expression of TGF-β1 and VEGF.

Conclusions Cryoinjury of detrusor induces DU/UAB with functional impairment lasting for up to 4 weeks, but the associated molecular changes are restored by 2 weeks. LESW improved bladder wall composition, and hastened functional recovery from cryoinjury.

Keywords Detrusor underactivity · Shock wave · Cryoinjury

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Introduction

The 2002 International Continence Society standardization report has defined Detrusor underactivity (DU) as a detrusor contraction of inadequate strength and/or duration resulting in prolonged bladder emptying and/or a failure to achieve complete bladder emptying in the absence of urethral obstruction [1]. DU contributes to the lower urinary tract symptoms (LUTS) and the clinical presentation is also called underactive bladder (UAB) [1, 2]. The prevalence of DU/UAB increases with age, which is 9–28% in young men (< 50 years), and is up to 48% in the elderly men (> 70 years) with non-neurogenic LUTS [3].

The etiopathogenesis of DU/UAB is linked to aging, bladder outlet obstruction, and diabetes mellitus with contribution of myogenic and neurogenic factors. Detrusor contractile strength generally decreases with age presumably due to degenerative changes in both muscle and nerve axons [4, 5]. Degenerative changes in detrusor smooth muscle are characterized by disrupted myocytes and increased collagen deposition, which leads to poor force generation and impaired response to acetylcholine and ATP [3].

There is a lack of effective treatment for DU/UAB, which motivates the development of reliable animal models to examine potential new therapies for DU/UAB. A previous mice study demonstrated that temporary freezing of the posterior bladder wall provokes cryoinjury, which elicits the sequelae of ischemia and reperfusion [6]. Cryoinjury leads to the destruction of smooth muscle layers and poor response to contractile stimuli [6, 7]. Cryoinjury of rat bladder wall has also been shown to produce detrusor thinning and reduce the amplitude of detrusor contraction at week 1 and 2 post cryoinjury [8]. We previously reported that one episode of low-energy shock wave (LESW) treatment can partially reverse the functional impairment of the bladder function provoked by cryoinjury [8]. LESW has been demonstrated to enhance gene expression related to angiogenesis and proliferation, and improve tissue neovascularization and regeneration, which has clinical utility in treatment of bone fracture, wound healing, myocardial infarction, and erectile dysfunction [9].

The current study investigated the long-term functional changes of cryoinjured bladder from 1 week to 4 weeks, and therapeutic effects elicited by multiple applications of LESW.

Methods

Animals

In all, 56 Sprague–Dawley rats (female, 250–300 g) were used. Experimental protocols were approved by the Institutional Animal Care and Use Committee of the Kaohsiung Chang Gung Memorial Hospital.

Induction of cryoinjured urinary bladder

An incision was made in lower abdomen under anesthesia with 2% halothane to expose the bladder filled with 1 ml saline. An aluminum rod of 8 mm diameter chilled on dry ice was placed bilaterally against the serosal surface of the bladder wall for 30 s, as described in previous report [8]. Sham group was exposed to room temperature aluminum rod for 30 s.

Bladder contractility (*N* = 5 for each group, total 20)

Control, 1, 2, and 4 weeks after applying cryoinjury, full-thickness longitudinal strips (10×2 mm^2) weighing about 20 mg were obtained from the injury site of bladder. Each bladder strips was placed in the organ bath containing 25 mL PSS solution (mmol/l: NaCl 130, KCl 4.7, $CaCl_2·(H_2O)$ 1.6, $MgSO_4·(H_2O)_7$ 1.17, $NaHCO_3$ 14.9, KH_2PO_4 1.18, glucose 5.5, $CaNa_2$-ethylenediaminetetraacetic acid 0.03, all from Sigma-Aldrich) aerated with a mixture of 95% O_2 + 5% CO_2 and maintained at 37 °C. The one end of bladder strip was fixed to an L-shaped hook at the bottom of the bath side and the other end was connected to an isometric tension transducer (PanLab, Spain) via a placed silk-snare. The concentration–response curves of bladder smooth muscle strips in response to different doses of KCl or carbachol were measured [10]. The contractile response was expressed as percent of 300 mM KCl-induced mean contractility of the control group in each experiment. All data are presented in Mean ± SEM.

Micturition Pattern in Metabolic Cage (*N* = 6)

The metabolic cage micturition pattern was assessed for 12-h 1 day before and at 1, 2, and 4 weeks after cryoinjury. Collected urine was measured by pan lying over a force transducer, which measured the change in weight of pan over time and send digitized signals to PowerLab. At the end of 4 weeks of metabolic cage study, all of the 6 animals were used for awake cystometrogram (CMG).

Awake free moving CMG (6 animals per group, *N* = 24)

CMG was performed on sham control animals and at the end of 1, 2, or 4 weeks after cryoinjury, by inserting a PE-50 tube into the bladder dome under halothane anesthesia as previously described for sensing pressure [8]. The intercontraction interval (ICI), amplitude (AMP), pressure threshold (PT), and pressure baseline (PB) were recorded. Measurements in each animal represented the average of 3 to 5 bladder contractions. Animal bladder was harvested at the end of CMG following transcardiac perfusion, as described previously [8]. Harvested bladder from each animal used for histology, immunohistochemistry, and Western blot analysis.

Low-energy shock wave (LESW) treatment (200 shocks, frequency of 2 pulses per second, and intensity 0.05 or 0.12 mJ/mm^2, 6 animals for each intensity group, *N* = 12)

The shock wave probe (Storz, Germany) was gently placed over the skin surface above the urinary bladder after application of ultrasound transmission gel during week 1 and 2 after cryoinjury under halothane anesthesia, as described previously [8]. The animals received twice-weekly LESW treatment for 2 weeks. At the end of week 2, awake CMG was performed.

Histology and Immunohistochemistry

Harvested bladder from different animal groups at time points was fixed in buffered 10% formaldehyde for 24–48 h, embedded in paraffin, and stained with hematoxylin and eosin or α-smooth muscle actin (SMA) (Abcam) or Ki-67 (Abcam), as described previously [8].

Western Blot Analysis from cryoinjured site for α-SMA, COX-2, TGF-β1, VEGF, CGRP, BDNF

The procedures for Western Blot analysis of cryoinjured bladder were as described previously [8]. Expression of α-SMA, COX-2, TGF-β1, VEGF, CGRP, and BDNF were analyzed according to the standard protocol (Amersham Biosciences).

Statistical analysis

Experimental results are presented as means ± SE and significance at 5% level was detected by Kruskal–wallis ANOVA followed by Dunn's test or Mann–Whitney *U* test.

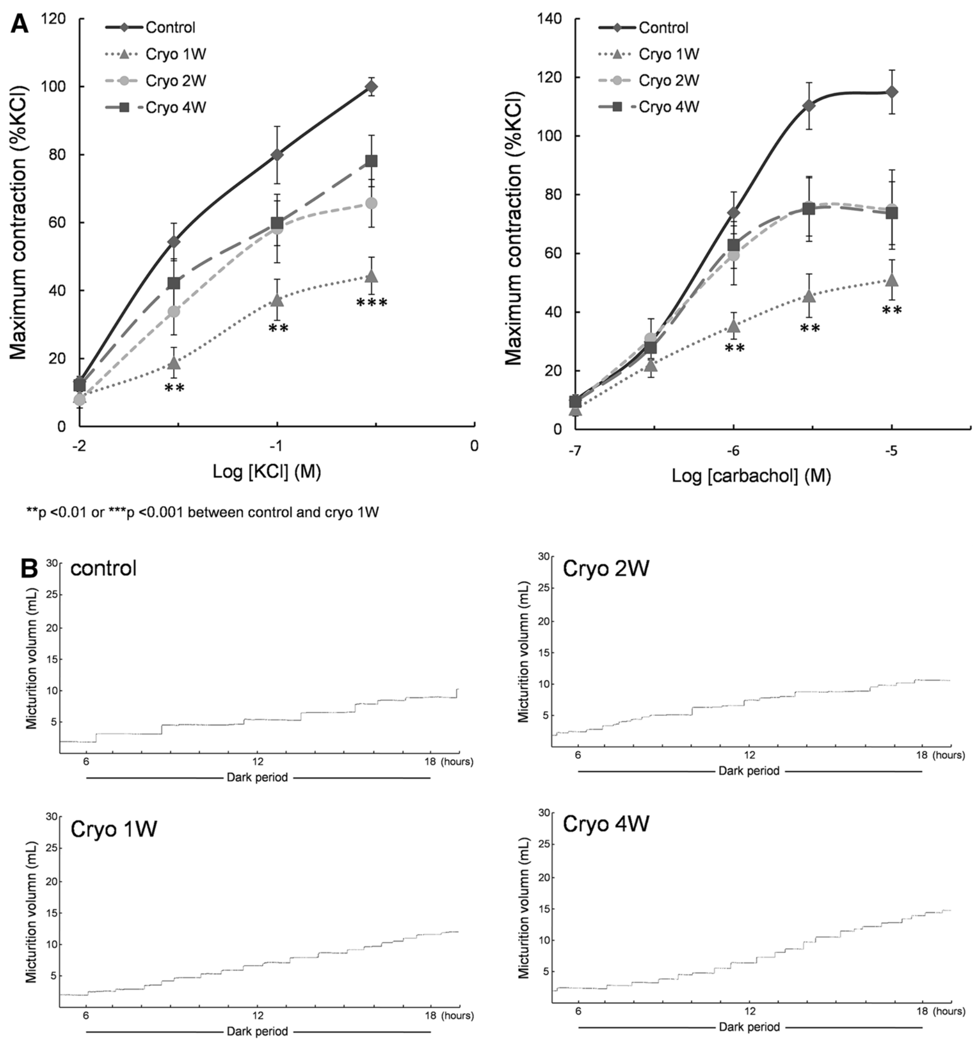


Fig. 1 **a** Effect of cryoinjury on the KCl or carbachol evoked contractions of the bladder strips from control, week 1, 2, and 4 post cryoinjury. In each experiment, contractile responses are expressed as percent of 300 mmole/l KCl-induced contraction. Left, KCl. Right, carbachol. Points represent mean ± SEM of 4 to 5 observations. Asterisk indicates significant difference between groups ($p < 0.05$). **b** Metabolic cage study demonstrated a normal micturition pattern before cryoinjury and progressive decrease of voiding volume at week 1 and week 2 post cryoinjury, which was partially recovered at week 4

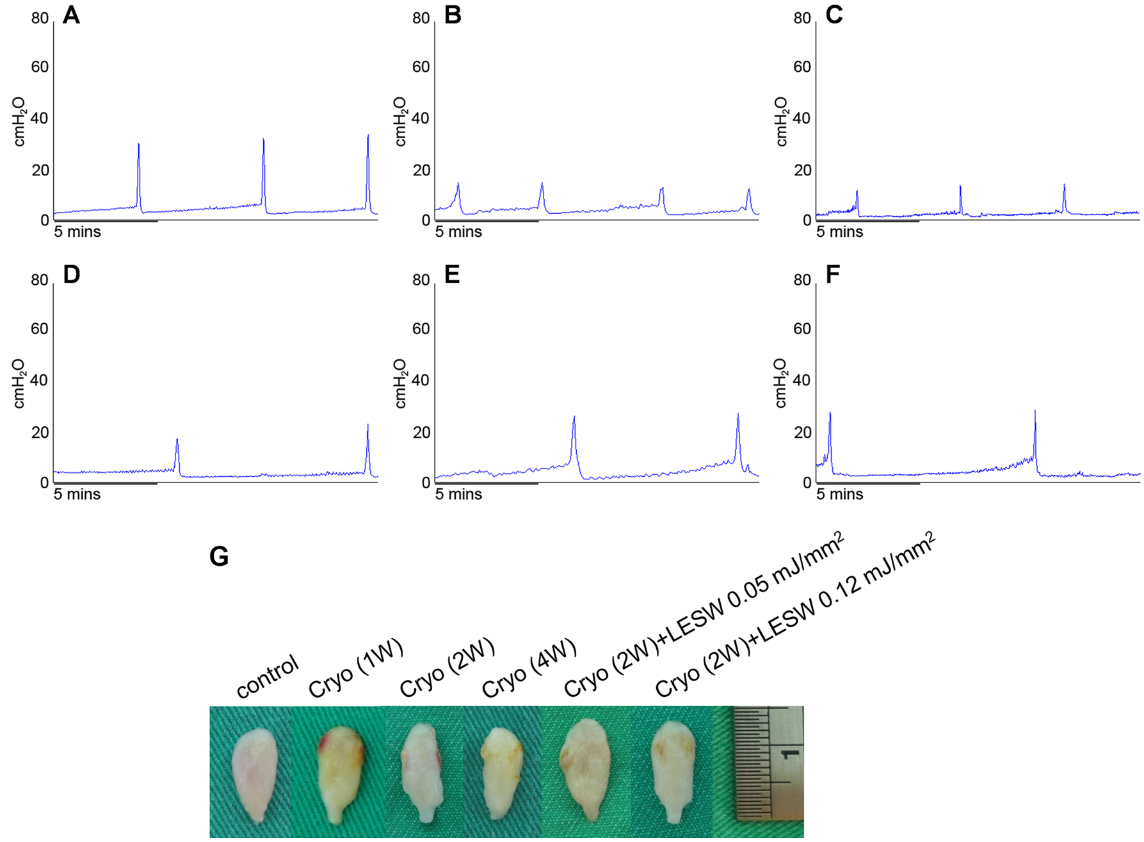


Fig. 2 Representative traces of in vivo continuous cystometrogram (CMG) in awake rats. CMG was performed in control (**a**), post cryoinjury at week 1 (**b**), week 2 (**c**), week 4 (**d**), post cryoinjury + LESW 0.05 mJ/mm^2 at week 2 (**e**), and post cryoinjury + LESW 0.12 mJ/mm^2 at week 2 (**f**). Compare to the control group, the contraction amplitude was significantly reduced post cryoinjury at week 1 and week 2, which was partially recovered at week 4, and by LESW treatment. **g** Bladder cross sectional view of normal control, and at week 1, week 2, week 4 post cryoinjury, and week 2 with LESW treatment. At the site of cryoinjury in urinary bladder, gross morphology revealed focal hemorrhage at week 1, which condition was resolution and replaced with scar tissue at week 2. The scar tissue was reduced at week 4 and post cryoinjury at week 2 with 0.12 mJ/mm^2 treatment

Results

Effects of cryoinjury on detrusor contractility

Figure 1A shows contractile responses to KCl, and carbachol of bladder smooth muscle strips from the 3 cryoinjured groups at week 1, week 2, and week 4 vs the normal control group. In this study, contractile responses to KCl and carbachol were normalized to the 300 mM KCl-induced contraction. Strips from 1-week post-cryoinjured group showed significant decrease in the contractile response to different concentrations of KCl or carbachol compared to the control group. This decrease in contractile responses to KCl or carbachol showed partial recovery at week 2 and week 4 post cryoinjury.

Effects of cryoinjury on micturition pattern

Metabolic cage studies on conscious rats revealed that volume of urine per void progressively decreased from 0.97 ± 0.22 ml at baseline to 0.78 ± 0.88 ml ($p = 0.7253$ vs. baseline) at week 1 and 0.54 ± 0.54 ml at week 2 ($p = 0.0684$ vs. baseline). This decrease in voided volume partially recovered by week 4 (0.66 ± 0.34 ml; $p > 0.9999$ vs baseline; Fig. 1b). The mean voiding frequency/12 h was 10.8 ± 8.4 measured at baseline, increased to 19.0 ± 9.4 ($p = 0.6373$ vs. baseline) at week 1, to 18.5 ± 10.9 ($p > 0.9999$ vs baseline) at week 2, and to 22.8 ± 10.6 ($p = 0.3124$ vs. baseline) at week 4.

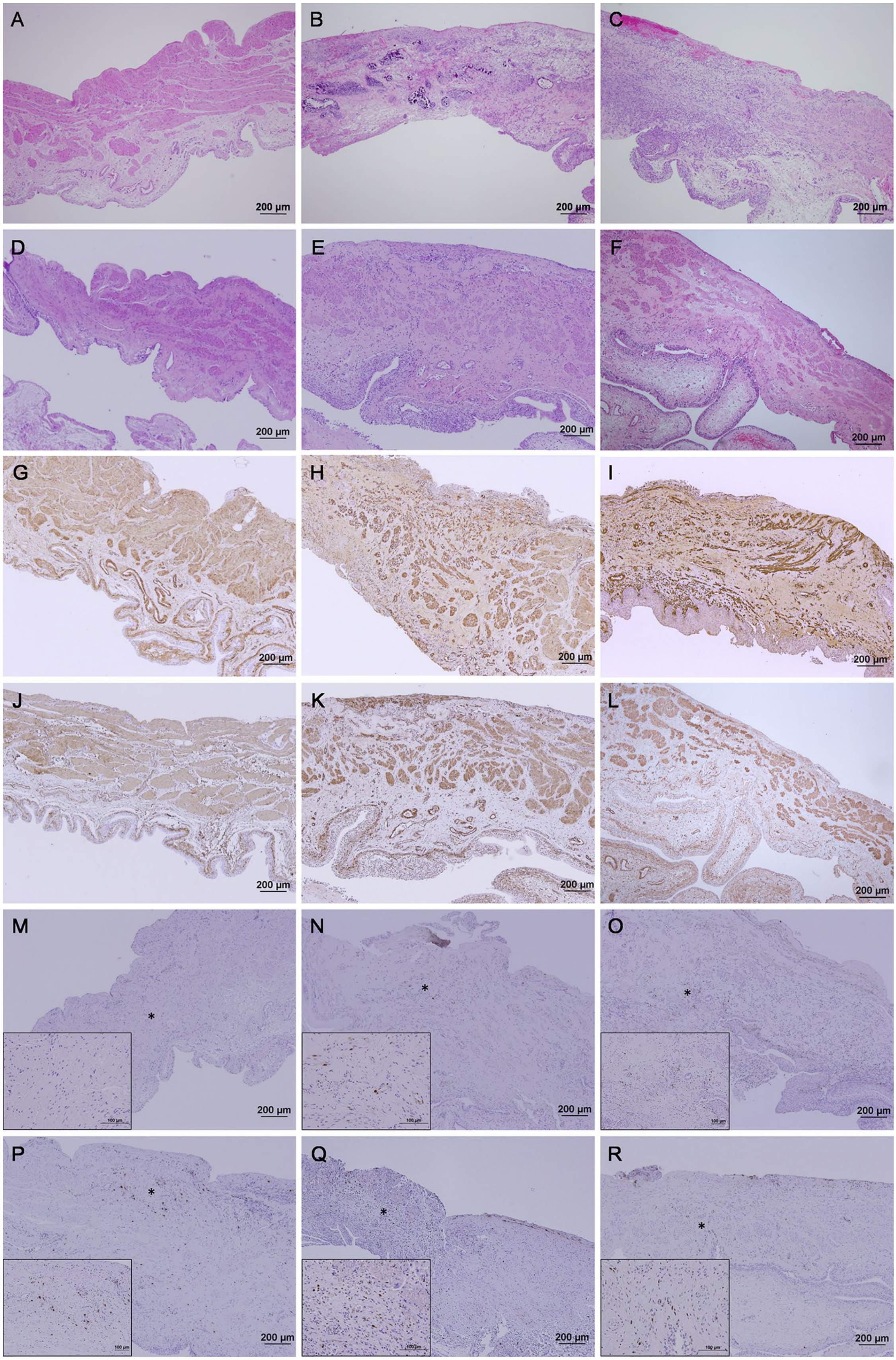
Tissue scarring in the bladder wall (Fig. 2g) consequent of cryoinjury is likely responsible for the increase in voiding frequency.

Table 1 Effects of cryoinjury and cryoinjury + low-energy shock wave (LESW) on CMG parameters

	N = 6				Adjusted *p* value (Dunn's multiple comparisons test)					
	Control	Cryo (1 week)	Cryo (2 week)	Cryo (4 week)	Control versus cryo (1 week)	control versus cryo (2 week)	Control vs cryo (4 week)	Cryo (2 wk) versus cryo (1 week)	Cryo (4 wk) versus cryo (1 week)	Cryo (4 wk) versus cryo (2 week)
ICI (min)	4.98 ± 1.62	4.80 ± 1.90	4.24 ± 2.32	9.49 ± 4.00	> 0.9999	> 0.9999	0.3003	> 0.9999	0.2131	0.0644
AMP (cm H_2O)	31.05 ± 4.10	13.96 ± 4.79	13.29 ± 4.13	17.73 ± 4.66	0.0066**	0.0031**	0.2243	> 0.9999	> 0.9999	0.9911
PB (cm H_2O)	1.82 ± 0.63	2.19 ± 0.46	2.61 ± 0.64	2.80 ± 1.69	> 0.9999	0.9186	> 0.9999	> 0.9999	> 0.9999	> 0.9999
PT (cm H_2O)	4.29 ± 3.11	5.22 ± 2.99	7.23 ± 3.32	4.43 ± 1.71	> 0.9999	0.4754	> 0.9999	> 0.9999	> 0.9999	0.7858

N = 6	Cryo (2 week)	Cryo (2 week) + 0.05 mJ/mm^2	Cryo (2 week) + 0.12 mJ/mm^2	Adjusted *p* value (Dunn's multiple comparisons test)		
				Cryo (2 week) + 0.05 mJ/mm^2 versus cryo (2 week)	Cryo (2 week) + 0.12 mJ/mm^2 versus cryo (2 week)	Cryo (2 week) + 0.12 mJ/mm^2 versus cryo (2 week) + 0.05 mJ/mm^2
ICI	4.24 ± 2.32	5.98 ± 3.43	8.75 ± 2.61	0.9913	0.0284*	0.3145
AMP	13.29 ± 4.13	22.07 ± 5.49	23.87 ± 4.35	0.0918	0.0125*	> 0.9999
PB	2.61 ± 0.64	3.52 ± 1.41	3.79 ± 2.95	0.7686	0.5833	> 0.9999
PT	7.23 ± 3.32	6.51 ± 3.48	5.11 ± 2.59	> 0.9999	0.7686	> 0.9999

Parameters included intercontraction interval (ICI), baseline pressure (BP), pressure threshold (PT), and amplitude (Amp). Data presented as means ± S.E.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◂**Fig. 3** Histology & Immunohistochemistry of normal control (**a, g, m**), post cryoinjury at week 1 (**b, h, n**), week 2 (**c, i, o**), week 4 (**d, j, p**), post cryoinjury + LESW 0.05 mJ/mm^2 at week 2 (**e, k, q**), and post cryoinjury + LESW 0.12 mJ/mm^2 at week 2 (**f, l, r**). Histology of bladder at week 1 and 2 post cryoinjury showed edematous changes in the lamina propria, marked thinning of the bladder wall with partial loss of urothelial lining of the mucosa and smooth muscle layer, which were partial recovered at week 4 post cryoinjury. Application of LESW fastened the recovery of the cryoinjury-induced histological changes as evinced by regenerated smooth muscle and proliferation of fibroblasts marked by increase in Ki-67 expression. **a–f** H&E stain; **g–l** SMA stain; Fig (M-R) Ki-67 stain. Magnification ×40. Inlet, magnification ×200

Effects of cryoinjury with or without LESW on CMG changes

As shown in Table 1 and Fig. 2, cryoinjury induces a significant decrease of contraction amplitude at week 1 (B, 55.0% decrease) and week 2 (C, 57.2% decrease) compared to the control group (A). Cryoinjury had no significant effects on any other CMG parameters. The decrease in contraction amplitude partially recovered itself in control group by week 4 (D), and the recovery is hastened to week 2 with LESW 0.05 mJ/mm^2 treatment (E) and LESW 0.12 mJ/mm^2 treatment (F). Treatment with LESW 0.12 mJ/mm^2 treatment prolonged the ICI, but other CMG parameters were not significantly altered.

Effects of cryoinjury with or without LESW on histological and immunohistological response

Necropsy of the cryoinjured site at the one week time point showed focal hemorrhage which resolved to produce scar tissue at week 2 (Fig. 2). The scar tissue after LESW 0.05 or 0.12 mJ/mm^2 treatment group at week 2 was similar to the cryoinjury group without treatment at week 4 indicating scar tissue formation is a natural healing response, which is hastened by LESW (Fig. 3). Histology of the cryoinjury group at the week 1 and week 2 post injury revealed tissue edema in the lamina propria, partial loss of the mucosa, and destruction of the smooth muscle. In contrast, histology of the bladder wall in the uninjured control group was unremarkable (Fig. 3). The effects of cryoinjury were partially reversed by week 4.

The Ki-67 staining progressively increased from 1.17 ± 0.54 baseline to 12.67 ± 7.00 at week 1 ($p = 0.9999$ vs. control), to 19.00 ± 5.48 at week 2 ($p = 0.1082$ vs control), and to 27.16 ± 10.95 at week 4 ($p = 0.0278$ vs. control). Progressive increase in the Ki-67 staining indicates the cell proliferation is a critical component of the healing response in bladder wall after cryoinjury (Fig. 4). LESW 0.05 mJ/mm^2 treatment increased the Ki-67 staining to 63.8 ± 18.7 at week 2 ($p = 0.0048$, vs. week 2 cryoinjury only), and 47.4 ± 12.54 at week 2 with LESW 0.12 mJ/mm^2 treatment ($p = 0.0207$, vs. week 2 cryoinjury only).

Effect of LESW on protein expression of α-SMA, COX-2, TGF-β1, VEGF, CGRP, BDNF

Western blotting (Table 2, Fig. 4) demonstrated that significant upregulation n of COX-2 at week 1, and TGF-β1 at week 1 and week 2, and downregulation of VEGF at week 1 and week 2, and CGRP at week 1 compared with control. There was a trend of decrease of α-SMA (control vs cryoinjury 1 week, 1: 0.2 ± 0.06, $p = 0.0816$). The protein expression was partially recovered at week 4. There was no significant change in BDNF.

LESW 0.12 mJ/mm^2 treatment also significantly increased VEGF compared with cryoinjury only group at week 2.

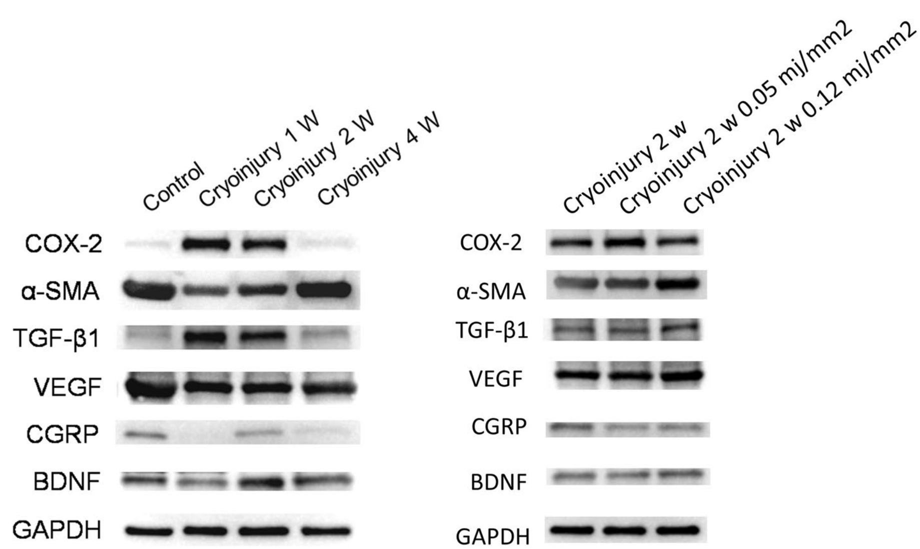

Fig. 4 Western blot for α-SMA, COX-2, TGF-β1, VEGF, CGRP, BDNF protein expression on the cryoinjured site with or without LESW. Significant upgrade expression of COX-2 at week 1, and TGF-β1 at week 1 and week 2, and downgrade expression of VEGF at week 1 and week 2, and CGRP at week 1 compared with control. There was a trend of decrease of α-SMA at week 1. The protein expression was partially recovered at week 4. There was no significant change of BDNF. Cryoinjury with LESW 0.12 mJ/mm^2 treatment has significantly increased TGF-β1and VEGF compared with cryoinjury only at week 2

Table 2 Effects of cryoinjury and cryoinjury + low-energy shock wave (LESW) on COX-2, α-SMA, TGF-β1, VEGF, CGRP, BDNF expression (band density)

$N = 6$	Control	Cryo 1W	Cryo 2W	Cryo 4W	Adjusted p value (Dunn's multiple comparisons test)					
					Control versus cryo 1W	Control versus cryo 2W	Control versus cryo 4W	Cryo 2W versus cryo 1W	Cryo 4W versus cryo 1W	Cryo 4W versus cryo 2W
COX-2	1	5.05 ± 0.84	2.79 ± 0.63	0.76 ± 0.0.09	0.0451*	0.8320	> 0.9999	> 0.9999	***0.0007	*0.0451
α-SMA	1	0.2 ± 0.06	0.77 ± 0.25	1.65 ± 0.32	0.0816	> 0.9999	> 0.9999	0.4618	**0.0015	0.3510
TGF-β1	1	5.4 ± 0.61	3.94 ± 0.33	2.73 ± 0.46	0.0001***	0.0240*	0.4618	> 0.9999	0.0816	> 0.9999
VEGF	1	0.7 ± 0.04	0.75 ± 0.05	0.88 ± 0.08	0.0065**	0.0140*	0.6788	> 0.9999	0.5495	0.8643
CGRP	1	0.4 ± 0.13	0.64 ± 0.16	0.52 ± 0.16	0.0165*	> 0.9999	0.5133	0.5433	> 0.9999	> 0.9999
BDNF	1	0.99 ± 0.0.04	1.55 ± 0.0.13	1.34 ± 0.0.2	> 0.9999	0.0914	0.8993	0.0052**	0.1143	> 0.9999

$N = 6$	Cryo 2W	Cryo 2W + 0.05 mJ/mm^2	Cryo 2W + 0.12 mJ/mm^2	Adjusted p VALUE (Dunn's multiple comparisons test)		
				Cryo 2W + 0.05 mJ/mm^2 versus cryo 2W	Cryo 2W + 0.12 mJ/mm^2 versus cryo 2W	Cryo 2W + 0.12 mJ/mm^2 vs cryo 2W + 0.05 mJ/mm^2
COX-2	1	1.06 ± 0.11	0.81 ± 0.12	> 0.9999	> 0.9999	0.3302
α-SMA	1	1.52 ± 0.25	1.53 ± 0.0.32	0.2956	0.5588	> 0.9999
TGF-β1	1	1.08 ± 0.15	1.33 ± 0.07	> 0.9999	0.0340*	0.1092
VEGF	1	1.11 ± 0.07	1.22 ± 0.0.06	0.5581	0.0246*	0.5581
CGRP	1	0.79 ± 0.14	1.05 ± 0.33	0.0765	0.1100	> 0.9999
BDNF	1	0.9 ± 0.08	1.07 ± 0.11	> 0.9999	> 0.9999	> 0.9999

Data presented as means ± S. E

Discussion

Chuang et al. demonstrated that cryoinjury on rat detrusor induced a decrease of contraction amplitude associated with muscle thinning at week 1 and 2 post cryoinjury [8]. This current study extends the previous study with the new finding that the detrusor function is partially recovered at week 4 post cryoinjury as evidenced by urodynamic study, muscle strip response to KCl and carbachol, and Ki-67 staining. Furthermore, they revealed a reduced expression of α-SMA, IL-6, and increased expression of COX-2 by western blotting from whole bladder specimens. The current study focused on the cryoinjured site only, where it found upregulated expression of COX-2, and TGF-β1, and downregulated expression of VEGF, CGRP, and α-SMA at week 1. The protein expression gradually recovered at week 4. Imamura et al. reported that 19 out of 84 growth factors mRNAs estimated by real-time RT-PCR arrays from the bladders of female nude mice 3 days post cryoinjury exhibited at least a twofold increase over the control bladders (including 2.85-fold increase for TGF-β1, and 2.33-fold increase for VEGFA) [6]. Contrary to the finding of whole bladder, our current results on western blot from injured site 1 week post cryoinjury found that TGF-β1 expression was 5.04-fold of control, but, VEGF was only 0.7-fold of control. These results suggest that effects of cryoinjury might not be localized to just the injured region and paracrine mediators may induce changes in the uninjured site.

The cryoinjured myogenic DU/UAB model has maximal characteristic of DU/UAB between week 1 and week 2 post cryoinjury, and bladder function partially recovers by 4 week. Therefore, we evaluated the therapeutic effects of LESW twice weekly on the current DU/UAB model at 2 week post cryoinjury. Although young rat (3–4 months old) can spontaneously recover from the harmful effects of cryoinjury by 4 weeks, we hypothesized that older rats (> 24 months old) may take longer in recovery and exhibit UAB symptoms for a prolonged time than young rats.

Wang et al. reported the therapeutic effect of once-weekly LESW (0.02 mJ/mm^2 at 3 Hz for 400 pulses) on the lower abdomen of the rats aiming toward the bladder and urethra in the streptozotocin-induced diabetic underactive bladder rat model [11]. The LESW treatment in DM group revealed significantly improved voiding function, enhanced detrusor contractility, increased muscle actin expression and muscle proportion of the bladder wall, and recovery of neuronal integrity and innervations [11]. In the previous study, the animals received shock wave treatment at multiple locations near the cryoinjured bladder wall immediately after cryoinjury of urinary bladder [8]. Chuang et al. found that LESW-induced cell proliferation and anti-inflammatory effect resulted in a dramatic improvement in the detrusor contractile strength [8]. Taken together, the therapeutic mechanism of LESW on restoration of bladder function in DU/UAB models might involves angiogenesis, nerve innervation, muscle regeneration, and muscle function restoration [12, 13].

The current study employed twice-weekly treatment of 200 shocks with the intensity of 0.05 or 0.12 mJ/mm^2 at 2 Hz on the abdominal skin over the urinary bladder at week 1 and 2. The transcutaneous method is more close to the potential use of LESW in human study. The cryoinjured animals with LESW treatment at higher energy level (0.12 mJ/mm^2) revealed a significant increase in Ki-67 staining, TGF-β1 and VEGF expression, and contraction amplitude compared with cryoinjured group at the end of week 2. The increase of ICI from 4.98 min at normal control group to 9.49 min at week 4 ($p = 0.3003$) post cryoinjury, and from 4.24 min post cryoinjury at week 2 to 8.75 min at the group of week 2 with LESW 0.12 mJ/mm^2 treatment ($p = 0.0284$), was an unexpected finding. It might be related to bladder remodeling and nerve regeneration after cryoinjury or LESW [11]. However, all of the above findings suggested that LESW at higher energy level improve the pace of recovery from cryoinjury in DU/IAB model.

These results underscore the promise of LESW to facilitate bladder wall regeneration and facilitate the recovery of voiding function in myogenic DU/UAB due to aging, bladder outlet obstruction, DM, or post-bladder cancer treatment. Since molecular changes at the cryoinjured site are distinct from the changes measured in the whole bladder, further studies are necessary to advance the understanding of this model in UAB.

It is worthwhile to state the obvious limitations of this study, which include lack of measurement of voided volume, voiding efficiency, and post-void residual (PVR) urine volume. All of these parameters are important factors for the evaluation of DU. Although our previous report described that the mean PVR increased from 225.00 ± 42.80 µl measured in the control group to 447.71 ± 102.1 µl at 1 week following cryoinjury ($p = 0.0583$), cryoinjury-mediated rise in PVR of cryoinjured group was reversed by LESW treatment [8]. Here we investigated the local biochemical and functional changes in the bladder contributing to the PVR improvement by assessing the contractile responses to KCl and carbachol. We expect to examine the contractile response to electrical field stimulation to complete a comprehensive analysis of the cryoinjury-induced DU/UAB model, and show the significant improvement of muscle contractility after LESW treatment in bladder strip contraction studies at 1 and 2 weeks after cryoinjury to further confirm the effects of LESW treatment for myogenic DU in the future.

Conclusion

Cryoinjury of detrusor induced DU/UAB at week 1 and 2, and maintain the physiological characters of DU/UAB at week 4. Twice-weekly LESW for 2 weeks improved bladder function recovery. LESW remodeled bladder wall composition, activated detrusor regeneration, and enhanced bladder muscle contractile function. Further investigations are warranted for clinical application of LESW for human DU/UAB.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Experimental protocols were approved by the Institutional Animal Care and Use Committee of the Kaohsiung Chang Gung Memorial Hospital. The work was performed at Kaohsiung Chang Gung Memorial Hospital.

References

1. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U et al (2002) The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Neurourol Urodyn 21:167–178
2. Dewulf K, Abraham N, Lamb LE, Griebling TL, Yoshimura N, Tyagi P et al (2017) Addressing challenges in underactive bladder: recommendations and insights from the Congress on Underactive Bladder (CURE-UAB). Int Urol Nephrol 49:777–785
3. Osman NI, Chapple CR, Abrams P, Dmochowski R, Haab F, Nitti V et al (2014) Detrusor underactivity and the underactive bladder: a new clinical entity? A review of current terminology, definitions, epidemiology, aetiology, and diagnosis. Eur Urol 65:389–398. https://doi.org/10.1016/j.eururo.2013.10.015
4. Brierly RD, Hindley RG, McLarty E, Harding DM, Thomas PJ (2003) A prospective controlled quantitative study of ultrastructural changes in the underactive detrusor. J Urol 169:1374–1378. https://doi.org/10.1097/01.ju.0000055781.07630.aa
5. Elbadawi A, Yalla SV, Resnick NM (1993) Structural basis of geriatric voiding dysfunction. II. Aging detrusor: normal versus impaired contractility. J Urol 150:1657–1667. https://doi.org/10.1016/S0022-5347(17)35867-6
6. Imamura T, Yamamoto T, Ishizuka O, Gotoh M, Nishizawa O (2009) The microenvironment of freeze-injured mouse urinary bladders enables successful tissue engineering. Tissue Eng Part A 15:3367–3375. https://doi.org/10.1089/ten.TEA.2009.0038
7. Somogyi GT, Yokoyama T, Szell EA, Smith CP, de Groat WC, Huard J et al (2002) Effect of cryoinjury on the contractile parameters of bladder strips: a model of impaired detrusor contractility. Brain Res Bull 59:23–28. https://doi.org/10.1016/S0361-9230(02)00833-X
8. Chuang YC, Tyagi P, Wang HJ, Huang CC, Lin CC, Chancellor MB (2018) Urodynamic and molecular characteristics of detrusor underactivity in a rat cryoinjury model and effects of low energy shock wave therapy. Neurourol Urodyn 37:708–715. https://doi.org/10.1002/nau.23381
9. Wang HJ, Cheng JH, Chuang YC (2017) Potential applications of low-energy shock waves in functional urology. Int J Urol 24:573–581. https://doi.org/10.1111/iju.13403
10. Lee WC, Chien CT, Yu HJ, Lee SW (2008) Bladder dysfunction in rats with metabolic syndrome induced by long-term fructose feeding. J Urol 179:2470–2476. https://doi.org/10.1016/j.juro.2008.01.086
11. Wang HS, Oh BS, Wang B, Ruan Y, Zhou J, Banie L et al (2018) Low-intensity extracorporeal shockwave therapy ameliorates diabetic underactive bladder in streptozotocin-induced diabetic rats. BJU Int 122:490–500. https://doi.org/10.1111/bju.14216
12. Zissler A, Steinbacher P, Zimmermann R, Pittner S, Stoiber W, Bathke AC et al (2017) Extracorporeal shock wave therapy accelerates regeneration after acute skeletal muscle injury. Am J Sports Med 45:676–684. https://doi.org/10.1177/0363546516668622
13. Wang B, Zhou J, Banie L, Reed-Maldonado AB, Ning H, Lu Z et al (2018) Low-intensity extracorporeal shock wave therapy promotes myogenesis through PERK/ATF4 pathway. Neurourol Urodyn 37:699–707. https://doi.org/10.1002/nau.23380