



Extracorporeal shock wave therapy modulates miRNA expression, reduces inflammation, and improves liposome retention in a rat model of cyclophosphamide-induced cystitis – experimental studies

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Introduction

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is characterized by bladder pain, increased urinary urgency and frequency, and nocturia in the absence of infection or other identifiable causes^[1]. The pathophysiology of IC/BPS is not completely clarified, and treatments for IC/BPS remain an unmet medical need.

Low-Energy Shock Waves (LESW) with anti-inflammatory effects and improvement of tissue regeneration have been shown to relieve IC/BPS symptoms in humans^[2–4]. MicroRNAs (miRNAs) are known to be dysregulated in various inflammatory diseases, including IC/BPS, and may play a role in controlling inflammation and urothelial permeability^[5]. Mu-opioid receptor (MOR) agonists have been explored for their inhibitory effects on inflammation, and modulation of MOR could potentially alleviate IC/BPS symptoms^[6].

This study aimed to investigate the mechanisms by which LESW modulates bladder inflammation, including its association with miRNAs and MOR, in a rat cystitis model. Intravesical mucosal protective agents, such as liposomes and hyaluronic acid, have been used for the treatment of IC/BPS; however, instilled drugs may be limited by short residence times within the

HIGHLIGHTS

- Cyclophosphamide injection induced bladder inflammation, an increased expression of the μ -opioid receptor, miR-21-5p, miR-146b-5p, IL-6, IL-1 β , and a decreased expression of ZO-1, which effects were partially reversed by low-energy shock waves (LESWs) treatment.
- LESW treatment increased the retention duration of liposomes within the bladder.
- LESW holds promise as a therapeutic approach for Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS), owing to its anti-inflammatory effects and ability to improve intravesical drug retention.

bladder. We hypothesized that LESW could prolong the therapeutic effects of intravesical drug therapy for IC/BPS by increasing drug retention within the bladder.

Methods

This study was approved by our Institutional Animal Care and Use Committee and was reported in accordance with the ARRIVE guidelines (Animals in Research: Reporting In Vivo Experiments)^[7]. Study 1: Female Sprague–Dawley rats were injected intraperitoneally with saline or cyclophosphamide (CYP, 200 mg/kg) to induce bladder inflammation. Subsequently, LESW (SD-1, Storz, Tägerwilen, Germany; 100 or 300 pulses, 2 pulses/s, 0.12 mJ/mm²) was applied over the skin surface above the bladder area under isoflurane anesthesia ($n=6$ for each group: control, CYP, CYP + LESW 100 shocks, CYP + LESW 300 shocks).

The bladders and L6 spinal cords were harvested on day 3 for histology (inflammatory grade of 0–3 as follows: 0; 1, mild; 2, moderate; and 3, severe)^[8]; immunostaining for MOR expression; western blotting for IL-6, IL-1 β , ZO-1, and E-cadherin expression; and RT-qPCR analysis of miR-21-5p and miR-146b-5p. Study 2: Following the completion of saline or CYP injection with or without LESW, 500 μ l of PBS or 500 μ l of CellVue NIR815 dye (Thermo, Waltham, Massachusetts, USA)-stained liposome (2 mg, Lipella, Pittsburgh, Pennsylvania, USA) suspension was instilled into the bladder ($n=3$ for each group). Bladder images of each group were taken with the IVIS Spectrum imaging system (PerkinElmer, Waltham, Massachusetts, USA) at

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0, 1, and 2 h, respectively. Experimental data are presented as mean $\pm$ standard deviation. Statistical analyses were performed using one-way analysis of variance with Tukey's post hoc test. Statistical significance was set at $P < 0.05$.

Results

The CYP-treated group showed a larger amount of inflammatory cell accumulation compared to the control group (Fig. 1). However, the inflammatory effects of CYP were significantly reduced by LESW treatment. These results indicate that CYP treatment induces inflammatory changes in the bladder and demonstrates the therapeutic effect of LESW on bladder inflammation.

As shown in Figure 2, CYP treatment induced an increase in MOR expression in the bladder mucosa region and L6 spinal cord. However, LESW treatment significantly reduced these effects, suggesting an interplay between bladder and spinal cord MOR expression in CYP-induced cystitis and demonstrating LESW's modulatory effects on MOR expression.

Western blotting demonstrated that IL-1 β and IL-6 protein levels were increased, and ZO-1 protein levels were decreased in the CYP-treated group compared to the control, and CYP effects

were partially reversed by LESW treatment (Fig. 3). These results suggest that CYP treatment induces changes in inflammatory cytokines and junctional protein, and the therapeutic effect of LESW on bladder inflammation might be related to modulation of inflammatory molecules and junctional protein.

Analysis of miRNAs revealed that CYP induced changes in miRNA expression within bladder tissue, primarily influencing the regulation of biological processes and cellular components (Supplement 1, Supplemental Digital Content 1, <http://links.lww.com/JS9/D140>). From the miRNA analysis dataset (Supplement 2, Supplemental Digital Content 1, <http://links.lww.com/JS9/D140>), two miRNA molecules, rno-miR-146b-5p and rno-miR-21-5p, associated with inflammatory responses were identified for further validation using qPCR. The results of qRT-PCR analysis showed significantly reduced expression levels of both rno-miR-146b-5p and rno-miR-21-5p in the bladder tissue of the LESW-treated group compared to the group treated with CYP (Supplement 3, Supplemental Digital Content 1, <http://links.lww.com/JS9/D140>). These results indicate that miRNAs are involved in bladder inflammation, and their effects can be mitigated by LESW treatment.

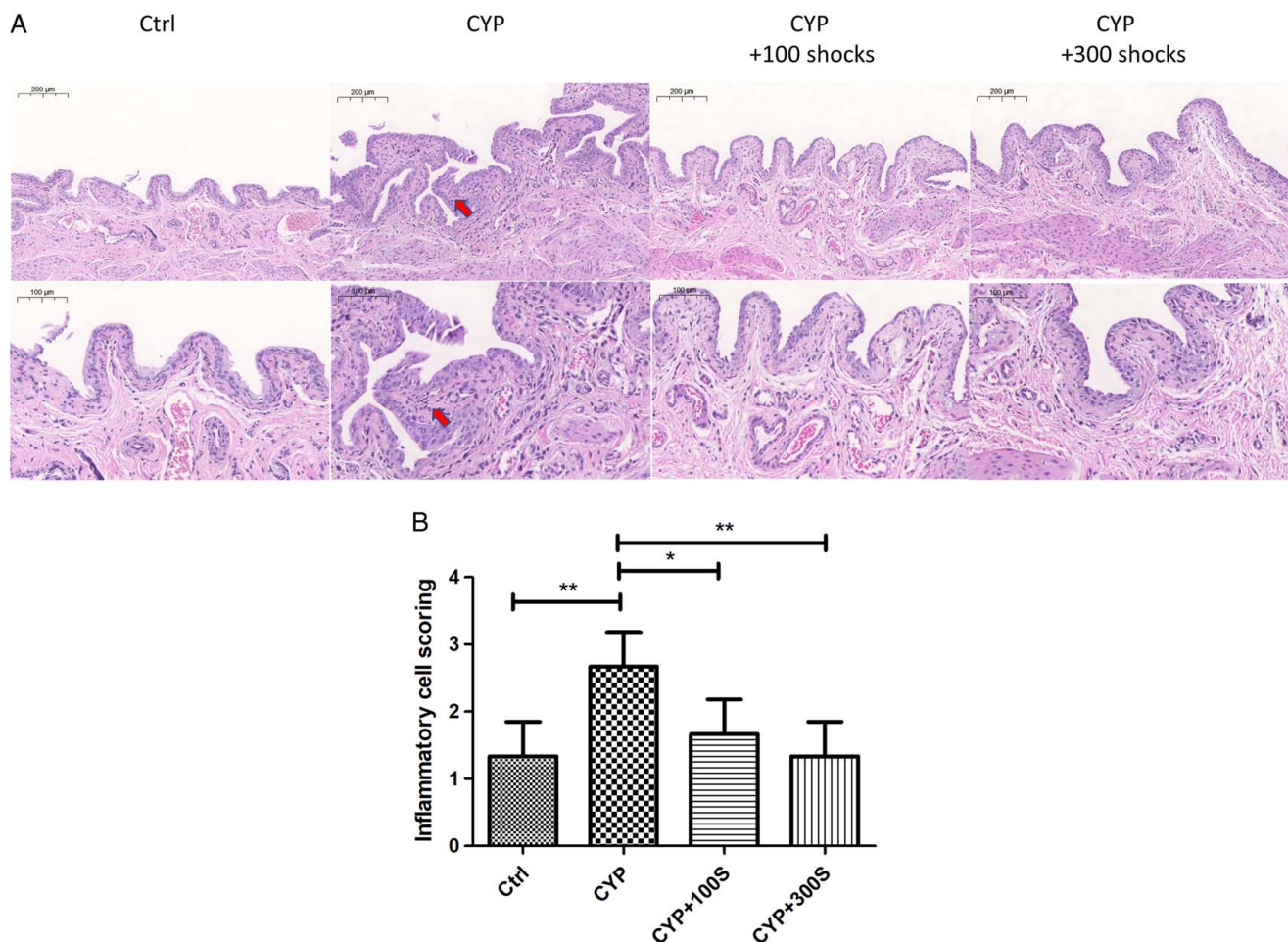


Figure 1. Hematoxylin and eosin (HE) staining of bladder tissue. HE staining of bladder tissue showed that CYP induced an increase in inflammatory cell infiltration, which was suppressed by LESW treatment. (upper panel – magnification $\times 50$; lower panel – magnification $\times 100$, Ctrl vs. CYP, $**P < 0.01$; CYP + 100S vs. CYP, $*P < 0.05$; CYP + 300S vs. CYP, $**P < 0.01$). CYP, cyclophosphamide. Arrow-indicates inflammatory cells.

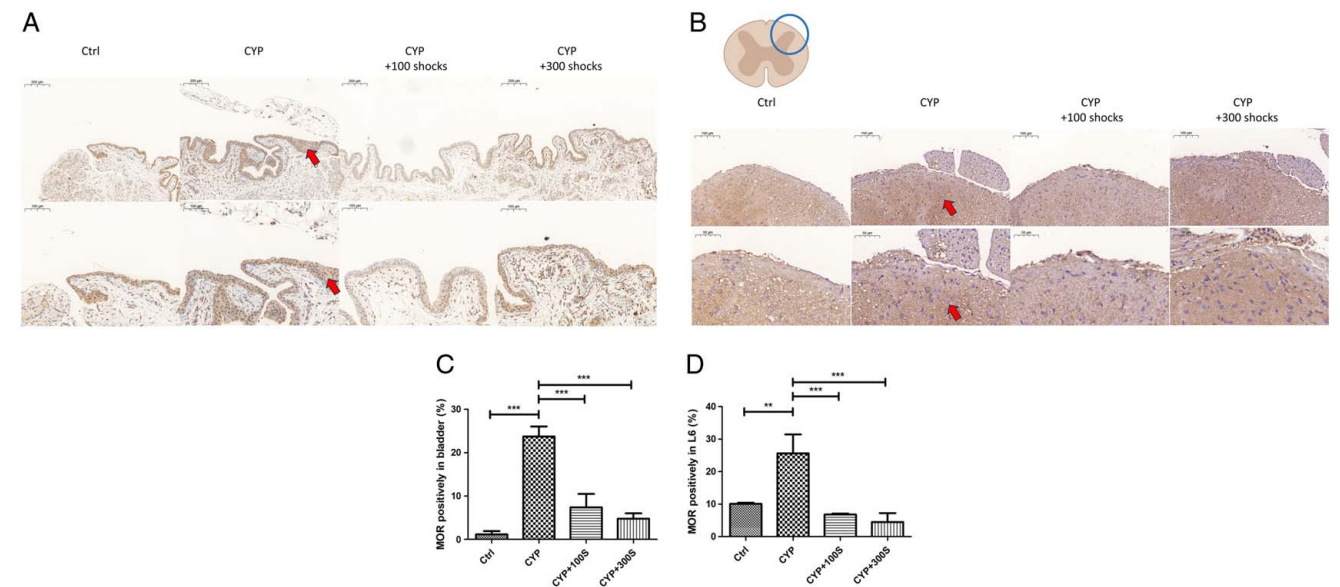


Figure 2. Mu-opioid receptors (MOR) staining of bladder and L6 spinal cord sections. On day 3, CYP treatment upregulated MOR expression in the urothelium and L6 spinal cord, the effects of which were decreased by low-energy shock wave (LESW) treatment. Magnification $\times 100$. (A) MOR staining in the bladder (upper panel – magnification $\times 50$; lower panel – magnification $\times 100$, arrow indicates positive area). (B) MOR staining in the L6 spinal cord (upper panel – magnification $\times 100$; lower panel – magnification $\times 200$, arrow indicates positive area). (C) Ctrl vs. CYP, CYP +100S vs. CYP, CYP +300S vs. CYP, *** $P < 0.001$. (D) Ctrl vs. CYP, ** $P < 0.01$, CYP +100S vs. CYP, CYP +300S vs. CYP, *** $P < 0.001$). CYP, cyclophosphamide. Arrow-indicates MOR staining positive area. Circular marker-indicate IHC presentation blocks.

The IVIS images revealed the presence of a fluorescent signal in the CYP plus LESW group after 2 h, whereas both the control and CYP-treated groups showed a decline in liposome fluorescence (Supplement 4, Supplemental Digital Content 1, <http://links.lww.com/JS9/D140>). These results suggest that LESW has the potential to extend the retention period of liposomes in the bladder.

Discussion

LESW has been shown to reduce bladder inflammation and pain in various preclinical studies^[9]. Our study extends these observations by demonstrating that LESW treatment can reduce the infiltration of inflammatory cells and upregulate tight junction proteins including ZO-1 and E-cadherin. Additionally, LESW

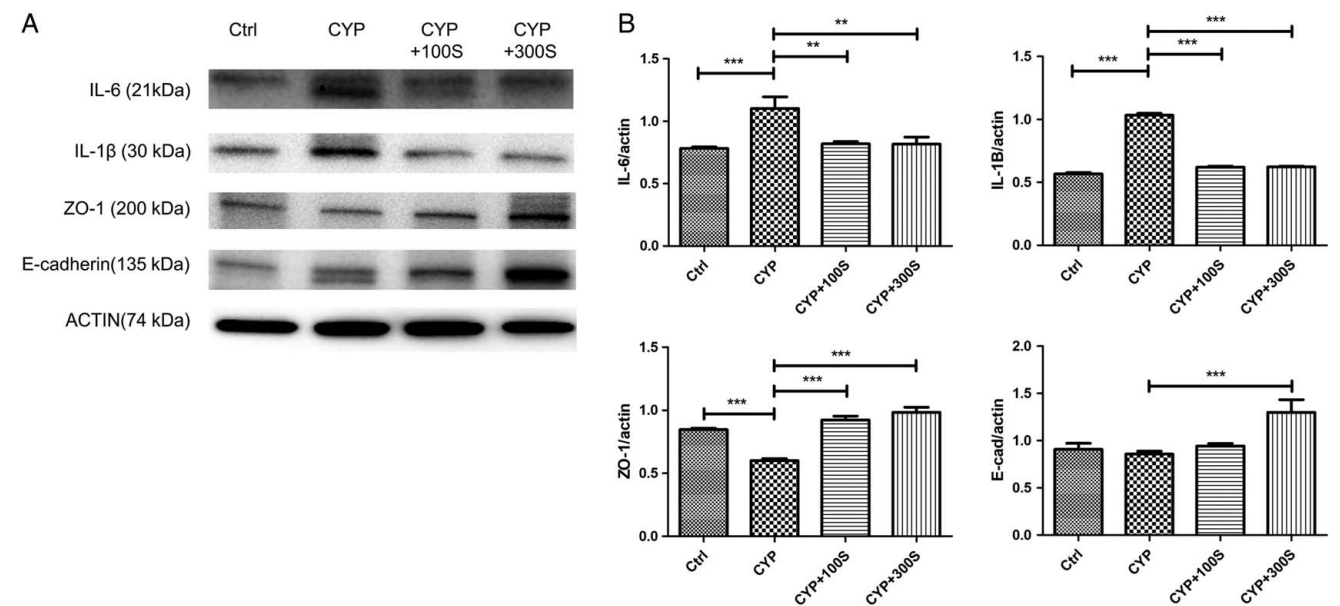


Figure 3. Western blot for IL-6, IL-1 β , and ZO-1 and E-cadherin expression in different groups. Compared to the control, the protein expression of IL-1 β and IL-6 was increased, while that of ZO-1 was decreased on day 3 post-CYP treatment. Low-energy shock wave (LESW) reverses IL-6, IL-1 β , and ZO-1 expression. Interestingly, CYP had no significant effect on E-cadherin expression, whereas LESW treatment further increased E-cadherin expression. CYP, cyclophosphamide.

lowered the expression of the inflammatory cytokines IL-1 β and IL-6. Furthermore, immunostaining of L6 and bladder tissues demonstrated a notable reduction in the expression of MOR in the LESW-treated group compared to the CYP-treated group. Moreover, miRNA-seq and RT-qPCR analyses revealed lower levels of miR-146b-5p and miR-21-5p in the LESW-treated group than those in the CYP group. We propose that LESW may alleviate inflammatory cytokine levels by modulating the MOR, associated miRNAs, and structural proteins, thereby reducing CYP-induced bladder inflammation. LESW has shown superior therapeutic effects in pain control compared to placebo for refractory IC/BPS; these effects were associated with changes in urinary cytokines^[3,4]. Our findings contribute to understanding the molecular mechanisms of LESW action in treating IC/BPS.

Furthermore, fluorescence imaging data from IVIS revealed that LESW may enhance liposome retention, potentially via the transient modulation of tight junction proteins, highlighting its potential to enhance the efficacy of intravesical drug delivery. This hypothesis is supported by previous research suggesting LESW's utility in boosting intravesical therapy effectiveness^[10].

Conclusion

Our findings suggest that LESW holds promise as a therapeutic approach for IC/BPS owing to its anti-inflammatory effects and ability to improve intravesical drug retention.

Ethical approval

This study was approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital (IACUC no. 2019121814).

Consent

Not applicable.

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Author contribution

W.Z. and C.Y.: conceptualization, methodology, writing – original draft; L.W. and W.H.: formal analysis, investigation, data curation; C.M., H.C., and C.Y.: review, editing, and supervision. All authors have contributed to the manuscript and have read and approved the final version of the manuscript.

Conflicts of interest disclosure

The authors declare no conflict of interest.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

Yao-Chi Chuang.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

Provenance and peer review

This is an invited manuscript. Guest Editor: Kandiah Raveendran. Theme: Shockwave treatment.

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