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Bae, Woong

Shin, Dongho

Piao, Jun

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Extracorporeal Shockwave Therapy Alleviates Inflammatory Pain by Down-Regulating NLRP3 Inflammasome in Experimental Chronic Prostatitis and Chronic Pelvic Pain Syndrome

Woong Jin Bae[1,2], Dongho Shin[1,2], Jun Jie Piao[1,2], Soomin Kim[1,2], Yong Sun Choi[1], Bong Hee Park[3], Hyun Jin Jung[4], Samuel Sorkhi[5], Saager Chawla[5], Chung Woon Cheon[6], Dae Up Kang[6], Jong Tae Choi[6], Sang-Hyuck Park[7], Sae Woong Kim[1,2,8], Mahadevan Raj Rajasekaran[5,9]

[1]Department of Urology, College of Medicine, The Catholic University of Korea, [2]Catholic Integrative Medicine Research Institute, College of Medicine, The Catholic University of Korea, Seoul, [3]Department of Urology, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Uijeongbu, [4]Department of Urology, Daegu Catholic University School of Medicine, Daegu, Korea, [5]Department of Urology, University of California San Diego Medical Center, San Diego, CA, USA, [6]Huons Meditech Co.,Ltd, Seongnam, Korea, [7]Institute of Cannabis Research, Colorado State University-Pueblo, Pueblo, CO, USA, [8]Green Medicine Co., Ltd, Busan, Korea, [9]San Diego Veterans Affairs Health Care System, San Diego, CA, USA

Purpose: To evaluate the anti-inflammatory and antioxidative effects of extracorporeal shockwave therapy (ESWT) on prostatitis and explore the mechanism of alleviating pain.

Materials and Methods: For *in vitro* testing, RWPE-1 cells were randomly divided into 5 groups: (1) RWPE-1 group (normal control), (2) LPS group (lipopolysaccharide inducing inflammation), (3) 0.1ESWT group (treated by 0.1 mJ/mm^2 energy level), (4) 0.2ESWT group (treated by 0.2 mJ/mm^2 energy level), and (5) 0.3ESWT group (treated by 0.3 mJ/mm^2 energy level). After ESWT was administered, cells and supernatant were collected for ELISA and western blot. For *in vivo* testing, Sprague-Dawley male rats were randomly divided into 3 groups: (1) normal group, (2) prostatitis group, and (3) ESWT group (n=12 for each). Prostatitis was induced by 17 beta-estradiol and dihydrotestosterone (DHT) administration. Four weeks after ESWT, the pain index was assessed for all groups and prostate tissues were collected for immunohistochemistry, immunofluorescence, apoptosis analysis and, western blot.

Results: Our *in vitro* studies showed that the optimal energy flux density of ESWT was 0.2 mJ/mm^2. *In vivo*, ESWT ameliorated discomfort in rats with prostatitis and inflammation symptoms were improved. Compared to normal rats, overexpressed NLRP3 inflammasomes triggered apoptosis in rats with prostatitis and this was improved by ESWT. TLR4-NFκB pathway was overactive after experimental prostatitis, compared to normal and ESWT groups, and prostatitis induced alterations in BAX/BAK pathway were inhibited by ESWT.

Conclusions: ESWT improved CP/CPPS by reducing NLRP3 inflammasome and ameliorated apoptosis *via* inhibiting BAX/BAK pathway in a rat model. TLR4 may play a key role in bonding NLRP3 inflammasome and BAX/BAK pathways. ESWT might be a promising approach for the treatment of CP/CPPS.

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Correspondence to: Mahadevan Raj Rajasekaran https://orcid.org/0000-0003-4188-1628
Department of Urology, University of California San Diego Medical Center, 3350, La Jolla Village Dr., San Diego, CA 92161, USA.
Tel: +1-858-552-8585 (X 7114), **Fax:** +1-858-552-7436, **E-mail:** mrajasekaran@health.ucsd.edu
Correspondence to: Sae Woong Kim https://orcid.org/0000-0002-9127-9400
Department of Urology, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Korea.
Tel: +82-2-2258-6226, **Fax:** +82-2-599-7839, **E-mail:** ksw1227@catholic.ac.kr

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Keywords: Apoptosis; Extracorporeal shockwave therapy; Inflammasomes; Prostatitis

INTRODUCTION

Chronic prostatitis and chronic pelvic pain syndrome (CP/CPPS) is a refractory male disease that has bothered patients and confused urologists for a long time [1]. Usual first-line therapies fail to elicit a satisfactory outcome in all patients [2]. Therefore, an effective alternative treatment is absolutely imperative.

An independent study suggested that widespread inflammation of the prostate was the vital pathogenic factor in CP/CPPS [3]. Previous reports implicated that NOD-, LRR-, and pyrin domains-containing protein 3 (NLPR3) inflammasome was expressed at the beginning of inflammation and promoted the inflammatory process [4]. Overexpressed NLPR3 inflammasome is believed to induce apoptosis by activating apoptosis-associated speck-like protein with a caspase-recruitment domain (ASC) and caspase-1[5]. Based on these reports, we propose that novel strategies to decrease NLPR3 inflammasome in the prostate may play an important role in attenuating for CP/CPPS. The correlation between BAX/BAK pathway and apoptosis has been explored previously reports. But the relationship between NLPR3 inflammasome and BAX/BAK pathway is still unclear during inflammation. Since apoptosis was simultaneously impacted by NLPR3 inflammasome and BAX/BAK pathway, we aimed to study the interaction between these two factors.

Recently, extracorporeal shockwave therapy (ESWT) has evolved as a novel and promising treatment approach in several medical fields [6]. In trauma repair, researchers found ESWT could accelerate cell proliferation and angiogenesis by stimulating more VEGF expression [7]. In addition, in the regeneration of peripheral nerves after injury, ESWT exhibited beneficial therapeutic outcome as well [8]. In our recent study, we found that ESWT improved erectile dysfunction (ED) effectively by activating PI3K/AKT/mTOR and NO/cGMP pathway [9]. In another study, researchers found that ESWT reduced the inflammatory responses and promoted recovery by down-regulating the expression of pro-inflammatory factors [10]. Although ESWT has now been clinically used for patients with CP/CPPS, the anti-inflammatory mechanism or the optimal energy flux density of ESWT for CP/CPPS is still unclear.

In this study, we propose to test a novel hypothesis that ESWT mediated anti-inflammatory effects in CP/CPPS by down-regulating NLPR3 inflammasome and improved apoptosis by inhibiting BAX/BAK pathways. We also determined the optimal energy flux density of ESWT and explored the mechanism of this anti-inflammatory and antiapoptotic effects using an experimental model of rat prostatitis model.

MATERIALS AND METHODS

1. *In vitro* Cells culture

RWPE-1 (ATCC) cells were cultured in low glucose-containing Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 20% fetal bovine serum (FBS; Gibco) and 5 ng/mL basis fibroblast growth factor (bFGF; Cell Signaling Technology) at 37 ℃ at 5% CO_2. Non-adherent cells were removed after 2 days and fresh culture medium was added. The culture medium was changed every 2 days. Cells were passaged when they reached approximately 90% confluence.

2. Effect of *in vitro* ESWT exposure to RWPE-1 cells

RWPE-1 cells were randomly divided into 5 groups: (1) RWPE-1 group (normal control), (2) LPS group (lipopolysaccharide inducing inflammation), (3) 0.1ESWT group (inflammatory RWPE-1 cells treated by 0.1 mJ/mm^2 ESWT), (4) 0.2ESWT group (inflammatory RWPE-1 cells treated by 0.2 mJ/mm^2 ESWT), and (5) 0.3ESWT group (inflammatory RWPE-1 cells treated by 0.3 mJ/mm^2 ESWT). Cells in groups 2, 3, 4, and 5 were treated by LPS (10 μg/mL). Twenty-four hours after LPS was added, cells in each group received different managements according to the project. An ESWT electromagnetic medical device, IMPO 88 (Huons Meditech) (Fig. 1), was used in this experiment as we previously described [11]. ESWT was administered for 15 minutes every day. Four hours after the third ESWT treatment, cells in

Fig. 1. The picture of IMPO 88 (Huons Meditech).

all groups were collected for Western blot and immunofluorescence studies.

3. *In vivo* experimental animal study design

All animal experiments in this study were approved by the Institutional Animal Care and Use Committee of the Catholic University of Korea (CUMC-2020-0100-01). All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA). Thirty-six male Sprague-Dawley rats (age, 8 weeks) were randomly divided into 3 equal groups: (1) control group, (2) prostatitis groups, and (3) ESWT group (12 rats in each group). Rats in the prostatitis group and ESWT group were administered 17 beta-estradiol and DHT for 4 weeks. Rats in the ESWT group were subjected to ESWT after model establishment and rats in the control or prostatitis group were only subjected to sham treatment.

4. ESWT administration to rats

Rats in ESWT groups were treated with ESWT for 4 weeks. Rats were anesthetized, and a shockwave applicator was placed on the abdomen. A total of 300 shocks were delivered at an energy flux density of 0.2 mJ/mm^2 and a frequency of 120 shocks/min. ESWT was repeated 3 times per week with a day's break.

5. Pain index assessment

Before and after ESWT treatment, pain index was evaluated for all rats by von Frey filaments (Stoelting Co.) and Dynamic Plantar Aesthesiometer (DPA) system (Stoelting Co.) as previously described [12]. Rats in all groups were placed individually in a wire-mesh cage. First, we tested mechanical allodynia by von Frey filaments. Incremental stimulation by filament was administered to every rat. A paw withdrawal was defined as a positive response and the force corresponding to the filament was recorded. Each rat was subjected to 10 trials at every filament after a short interval. For the DPA test, a ramp of 2 g/s and a cut-off value of 30 g was set. Once the paw withdrawal appeared, the value was recorded and the needle automatically fell back into the starting position. Each rat was tested three times and the mean value was recorded as the threshold. After the last pain index assessment, rats were sacrificed and the prostate was harvested for histologic analysis and western blotting.

6. Immunohistochemistry and Immunofluorescence

The collected prostate samples were fixed in 4% paraformaldehyde for 24 hours at 4 ℃ before creating a paraffin block. Immunohistochemistry (IHC) and immunofluorescence stainings were performed as previously described [13]. RWPE-1 cells were immunostained with the VEGF (1:200; Santa Cruz Biotechnologies), actin (1:200; Abcam), and 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Inc.). The prostate paraffin sections were immunostained with the NLRP3 (diluted 1:200; Santa Cruz Biotechnologies), ASC (1:200; Santa Cruz Biotechnologies), actin (1:200; Abcam), and mounted with 4,6-diamidino-2-phenylindole (DAPI; Vector Laboratories, Inc.) to stain the nuclei.

7. TUNEL assay and H&E staining

Apoptosis in situ was analyzed by TUNEL assay using an In Situ Cell Death Detection Kit, TMR red (Roche Diagnostics GmbH), according to the manufacturer's protocols. The apoptotic index was expressed as the number of TUNEL and DAPI-positive cells in three randomly chosen sections of the prostate per animal. And prostates of all rats were stained by H&E staining with a H&E staining kit (Sigma) according to manufacturer's instructions.

8. Western blotting

RWPE-1 cells and rat prostate tissues were homogenized using ice-cold RIPA buffer (Cell Signaling Tech-

nology). The homogenized sample was then centrifuged at 12,000×*g* for 10 minutes at 4 ℃ and its supernatant was extracted. This supernatant was electrophoresed on NuPAGE 4%–12% bis-Tris gel (Invitrogen) and then transferred onto a nitrocellulose membrane. After the transfer, the membrane was blocked with 5% skim milk at room temperature for 1 hour and then incubated with the primary antibodies. After this, the membrane was incubated with a secondary antibody conjugated to horseradish peroxidase for 1 hour at room temperature. The enhanced chemiluminescence method (Amersham) was used for protein detection.

9. Image and statistical analysis

Digital images were obtained using a Zeiss LSM 510 Meta confocal microscope (Zeiss), and the mean intensity was calculated using ZEN 2009 (Zeiss). The images were quantified by Image J (Media Cybernetics). The results were analyzed using SPSS 20.0 software (IBM Corp.). The measurement data are presented as mean±standard deviation and the multi-group comparisons were made with (ANOVA) followed by the Tukey–Kramer test for post hoc comparisons. Data expressed as proportions were assessed with a chi square test. Values of $p<0.05$ were considered to indicate a statistically significant difference.

RESULTS

1. Assessment of *in vitro* optimal ESWT energy flux density ESWT

Based on our previous studies, we set an intensity gradient as 0.1 mJ/mm^2, 0.2 mJ/mm^2, and 0.3 mJ/mm^2. The results showed that at the energy of 0.2 mJ/mm^2, cells expressed the highest VEGF ($p<0.01$) compared to the LPS group. And as energy increased, VEGF expression *in vitro* was reduced inversely which suggested that excessive transmission of energy decreased cell

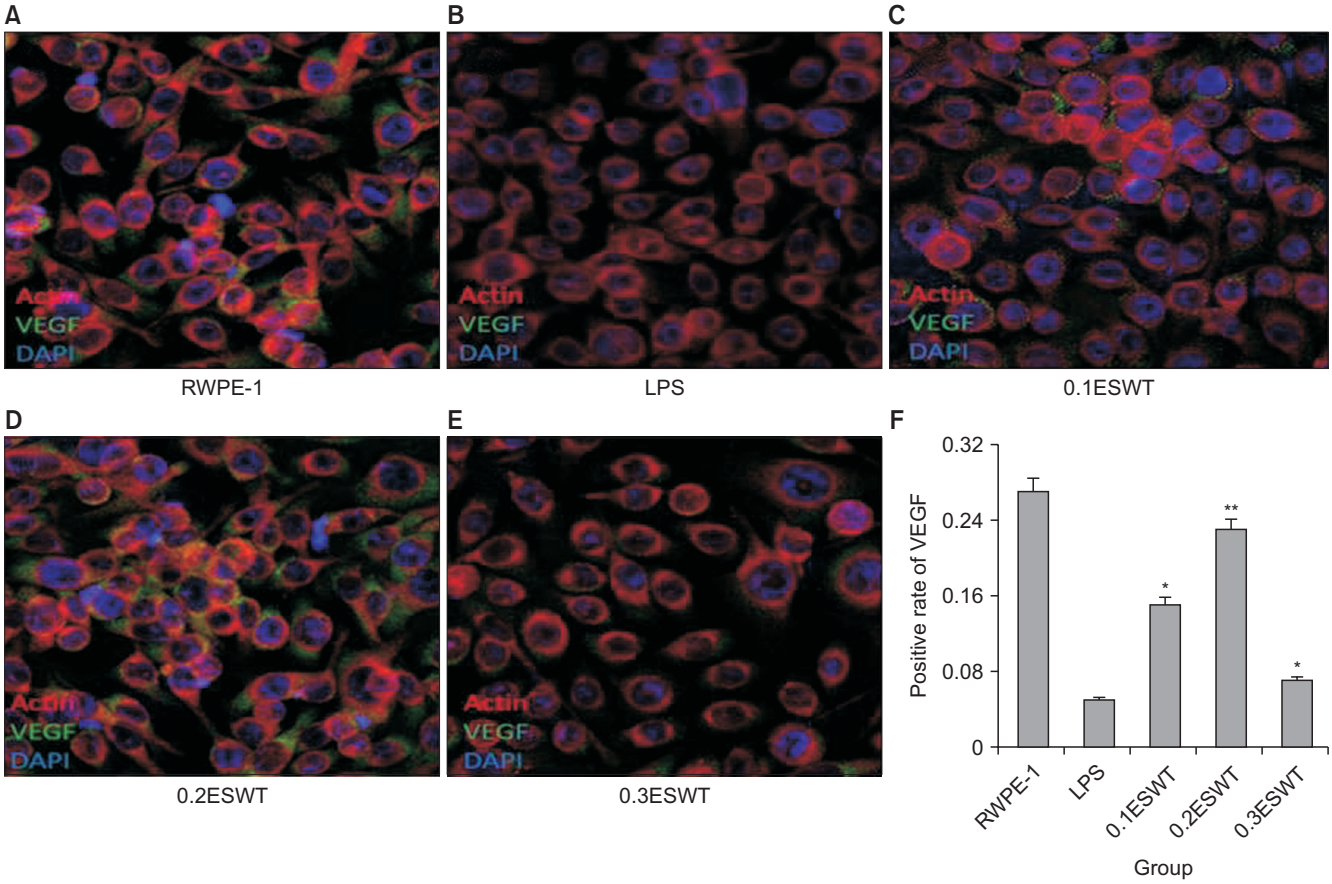

Fig. 2. (A-E) Representative images of VEGF staining for each group. Original magnification: ×200. (F) Positive rate of VEGF for each group. Each bar shows the mean values (standard deviation). ESWT: extracorporeal shockwave therapy, RWPE-1 group: normal control, LPS group: lipopolysaccharide inducing inflammation, 0.1ESWT group: treated by 0.1 mJ/mm^2 energy level, 0.2ESWT group: treated by 0.2 mJ/mm^2 energy level, 0.3ESWT: group treated by 0.3 mJ/mm^2 energy level. *$p<0.01$ compared with LPS group. **$p<0.01$ compared with 0.1ESWT group and 0.3ESWT group.

viability (Fig. 2). Meanwhile, for evaluating the cell viability under inflammation, we tested VEGF, HIF1, NF-κB, and COX-2 by western blot (Fig. 3). We found that only in 0.2ESWT group, the expression of all these proteins was higher when compared to the LPS group ($p<0.05$). HIF1/NF-κB pathways play a vital role during injured cell repair. In our experiment, we found that HIF1/NF-κB pathway was overactive which meant 0.2 mJ/mm^2 ESWT maximally stimulated cell repair function, instead of impacting normal cells. Meanwhile, COX-2 in the 0.2ESWT group was lower than in other groups ($p<0.05$), which meant inflammation was im-

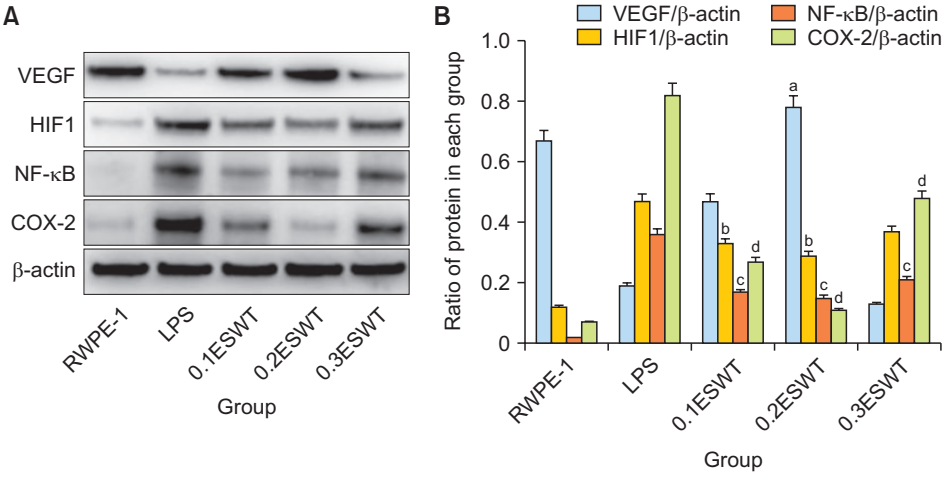

Fig. 3. (A) Representative western blot results in each group. (B) Quantity analysis of western blotting, including VEGF/β-actin, HIF1/β-actin, NF-κB/β-actin, and COX-2/β-actin. Each bar shows the mean values (standard deviation). ESWT: extracorporeal shockwave therapy, RWPE-1 group: normal control, LPS group: lipopolysaccharide inducing inflammation, 0.1ESWT group: treated by 0.1 mJ/mm^2 energy level, 0.2ESWT group: treated by 0.2 mJ/mm^2 energy level, 0.3ESWT group: treated by 0.3 mJ/mm^2 energy level. [a]$p<0.01$ compared with other groups. [b]$p<0.05$ compared with LPS group. [c]$p<0.05$ compared with LPS group. [d]$p<0.05$ compared with LPS group.

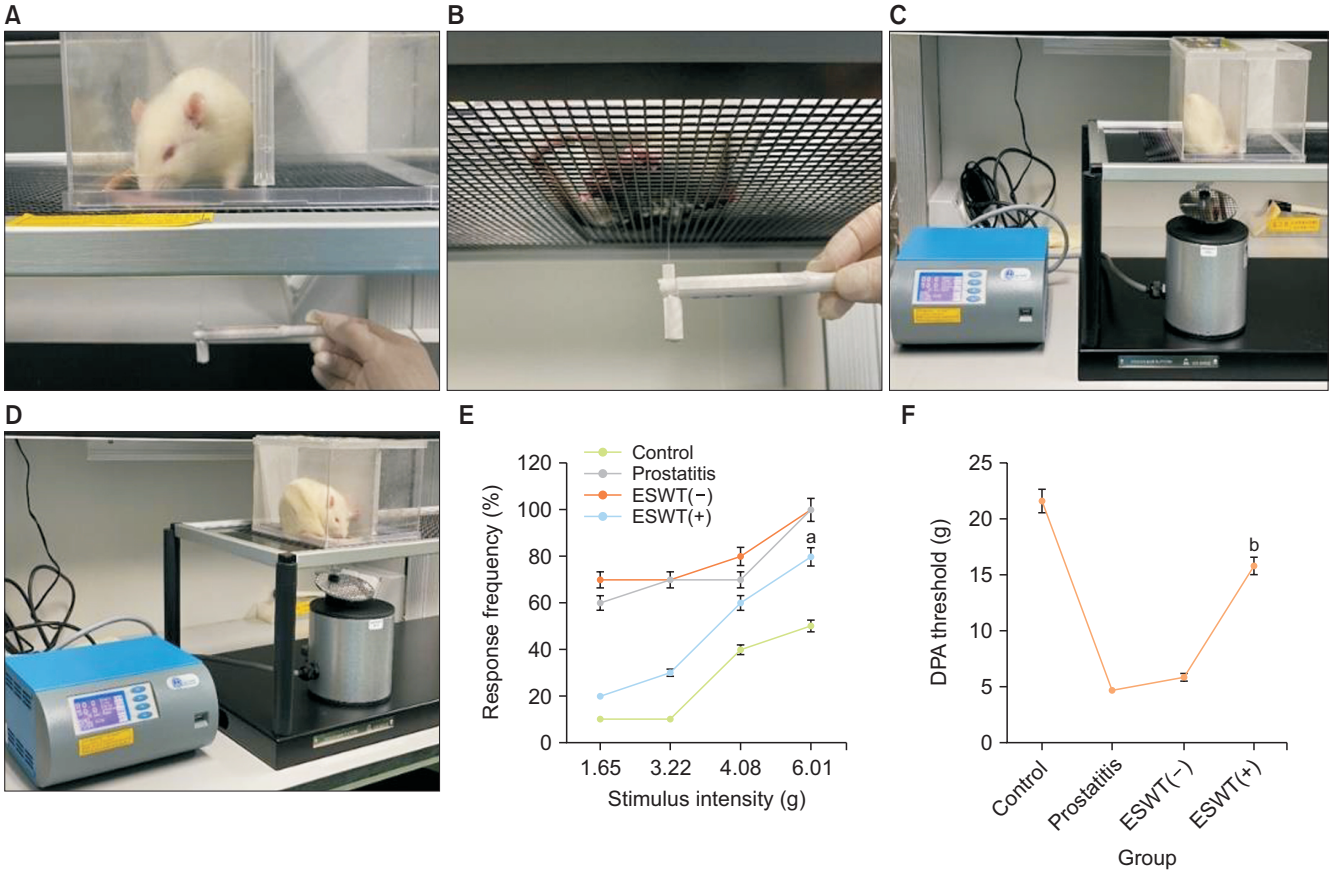

Fig. 4. (A, B) Representative images of von Frey filaments test. (C, D) Representative images of Dynamic Plantar Aesthesiometer (DPA). (E) Results of von Frey filaments in each group. ESWT: extracorporeal shockwave therapy. [a]$p<0.05$ compared with ESWT (-) group. (F) DPA results in each group. [b]$p<0.01$ compared with EWST (-) group.

proved obviously under 0.2 mJ/mm^2 ESWT. COX-2 in 0.3ESWT group was higher than in 0.1ESWT group, probably because over-high energy destroyed intrinsic cellular function and decreased cell activity.

2. Assessment of pain index in prostatitis rats after ESWT

For evaluating the effect of EWST on alleviating pain, we used von Frey filaments and DPA on rats in each group. In the ESWT group, pain threshold was tested before and after EWST administration, respectively. Our results (Fig. 4E) suggest that after ESWT, von Frey filaments response frequency was ameliorated, when compared to the pre-ESWT level ($p<0.05$). Fig. 4F showed that DPA threshold was increased by ESWT as well ($p<0.01$). These results indicate that pain due to prostatitis was distinctly improved by ESWT.

3. Evaluation of reduction of Inflammation with ESWT in rats

We studied the anti-inflammatory effect in the prostatitis model. H&E staining results revealed that 17 beta-estradiol and DHT induced tissue edema in rat with prostate symptoms, which was caused by inflammatory response in situ. After ESWT for 4 weeks, inflammatory edema showed a clear reduction (Fig. 5). For assessing the ESWT effect accurately, inflammatory factors including IL-1β and COX-2 were evaluated by western blot. The results (Fig. 6) showed that IL-1β and COX-2 protein levels were reduced by ESWT, which suggests inflammation was attenuated by ESWT in rats with symptoms of prostatitis. Combined with pain index and H&E staining, we conclude that ESWT could ameliorate inflammatory symptoms by reducing inflammatory mediators in rats with symptoms of prostatitis.

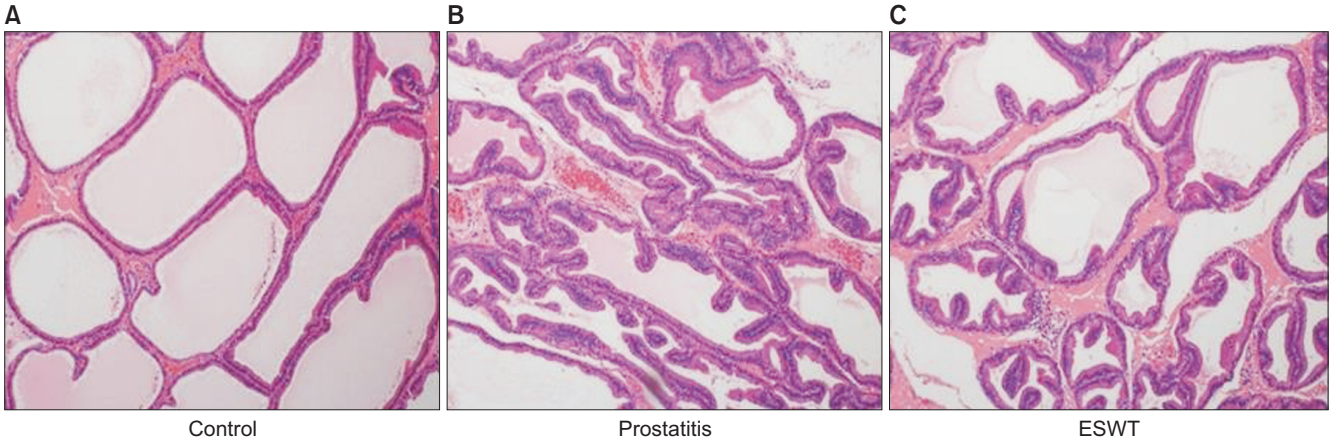

Fig. 5. Representative images of H&E staining for control group (A), prostatitis group (B), and ESWT group (C). Original magnification: ×100. ESWT: extracorporeal shockwave therapy.

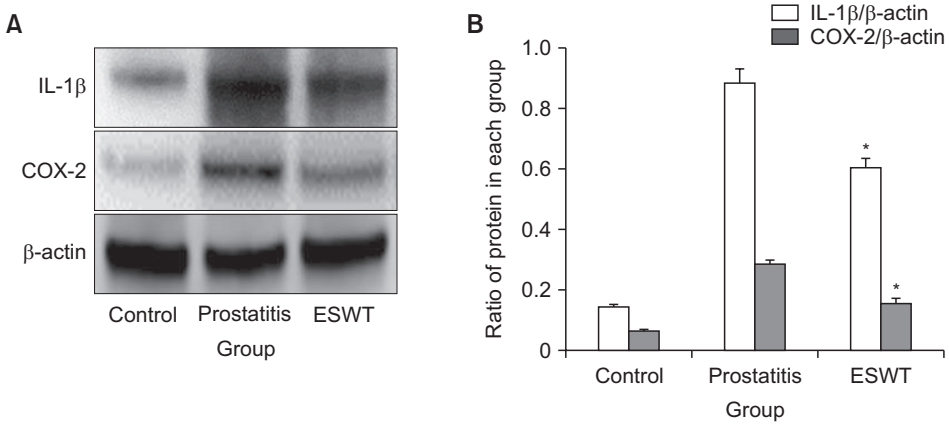

Fig. 6. (A) Western blot results of IL-1β and COX-2 for each group. (B) Quantitive analysis of western blot for IL-1β/β-actin and COX-2/β-actin. *$p<0.01$ compared to prostatitis group. Each bar shows the mean values (standard deviation). ESWT: extracorporeal shockwave therapy.

4. Evaluation of NLRP3 inflammasome and apoptosis

For assessing apoptosis in prostatitis, first, a TUNEL assay was performed in each group. Fig. 7C and 7D showed that apoptosis was increased in prostatitis, but after ESWT this phenomenon was moderated. Mean-

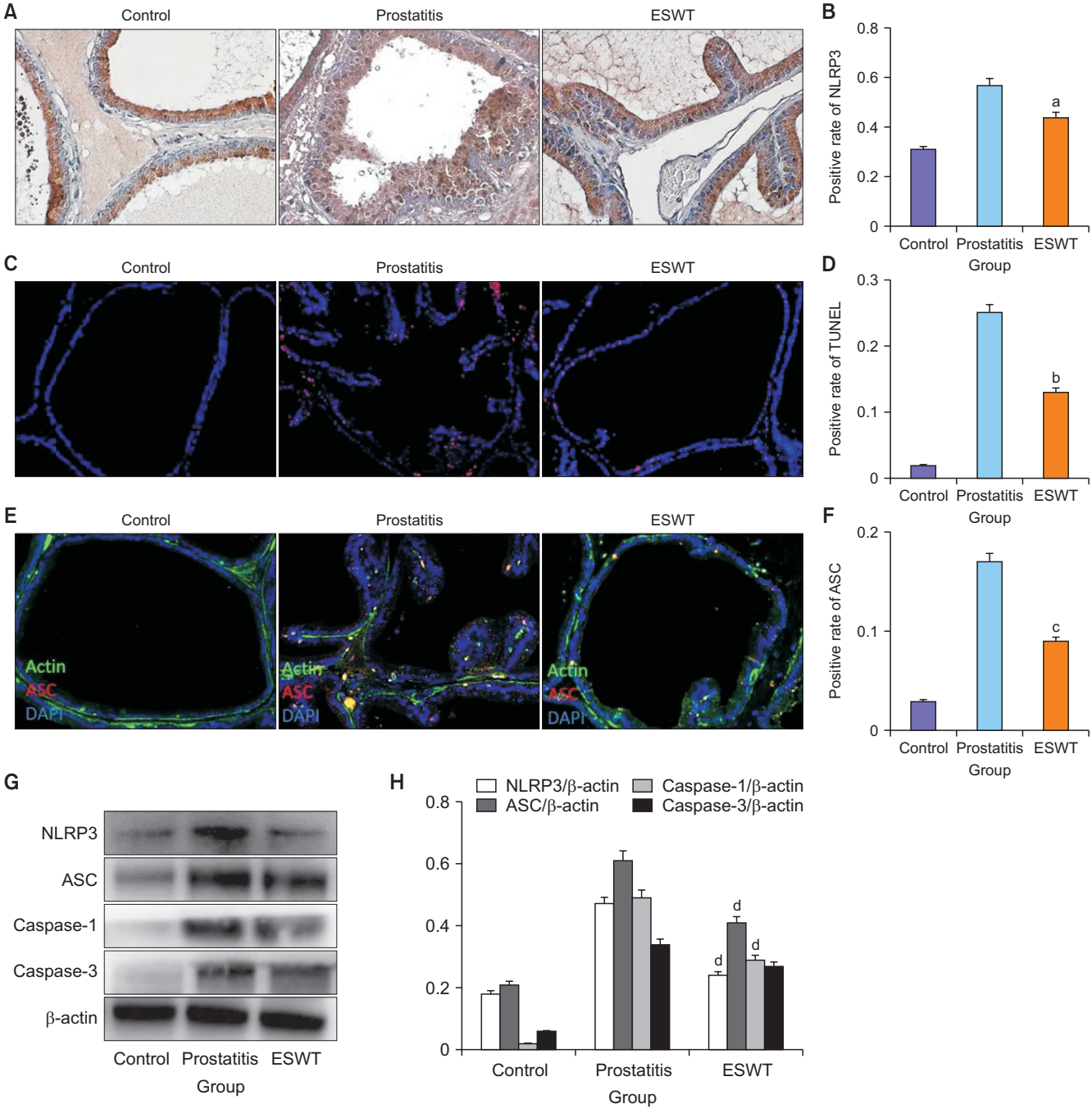

Fig. 7. (A) Representative images of immunohistochemistry (IHC) for each group. Original magnification: ×200. (B) Positive rate of NLRP3 for each group. Each bar shows the mean values (standard deviation). [a]p<0.05 compared with prostatitis group. (C) Representative images of TUNEL for each group. Original magnification: ×200. (D) Positive rate of TUNEL results for each group. Each bar shows the mean values (standard deviation). [b]p<0.05 compared with prostatitis group. (E) Representative images of ASC staining for each group. Original magnification: ×200. (F) Positive rate of ASC for each group. Each bar shows the mean values (standard deviation). [c]p<0.05 compared with prostatitis group. (G) Western blot results of NLRP3, ASC, caspase-1, caspase-3 and β-actin for each group. (H) Quantity analysis of western blot for NLRP3/β-actin, ASC /β-actin, caspase-1/β-actin, and caspase-3/β-actin. [d]p<0.05 compared with prostatitis group. Each bar shows the mean values (standard deviation). ESWT: extracorporeal shockwave therapy.

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while, caspase-3 a marker of apoptosis was measured by western blot [14,15]. Fig. 7G and 7H demonstrate that caspase-3 appeared to have a similar outcome to the TUNEL results, which further support our claim that apoptosis was improved by ESWT in prostatitis. Next, we focused on the inflammasome expression *in vivo*. IHC and western blot results (Fig. 7A, 7B, 7G, 7H) showed that NLRP3 inflammasome was overexpressed in prostatitis that was down-regulated by ESWT ($p<0.05$). In addition, as NLRP3 inflammasome components, the immunoreactivity of ASC and caspase-1 proteins were evaluated by immunofluorescence and Western blot as well [16]. The results (Fig. 7E, 7F, 7G, 7H) showed that after ESWT, the ASC and caspase-1 were also decreased in prostatitis. All the results in Fig. 7 provide support to our claim that ESWT improves apoptosis in prostatitis by inhibiting the overexpression of NLRP3 inflammasome.

5. ESWT inhibited BAX/BAK pathway and down-regulated TLR4-NFκB pathway *in vivo*

We explored the possible underlying mechanism of ESWT-mediated decrease in apoptosis. We found that BAX and BAK were overexpressed in prostatitis, and ESWT inhibited BAX/BAK pathway (Fig. 8A, 8B). Meanwhile, BCL-XL, as an inhibitor of BAX/BAK pathway, was detected as well. The result showed that ESWT could improve the expression of BCL-XL in prostatitis. So, we believed that ESWT inhibited BAX/BAK pathway by stimulating overexpression of BCL-XL.

In our previous study [16], we proved that micro external stimulus inhibited TLR4/NF-κB pathway, and then reduced inflammatory factors. So, we researched the effect of ESWT on TLR4/NFκB pathway. Western blot results (Fig. 8C, 8D) illuminated that TLR4 and NF-κB were down-regulated by ESWT. This result proved ESWT improved prostatitis as well.

DISCUSSION

Due to the high prevalence of CP/CPPS in middle-aged men, and its impact on the quality of life, numerous studies have sought to identify an ideal management strategy. However, it is now well-appreciated that the poorly understood pathophysiology of CP/CPPS makes targeted therapy challenging, leaving providers

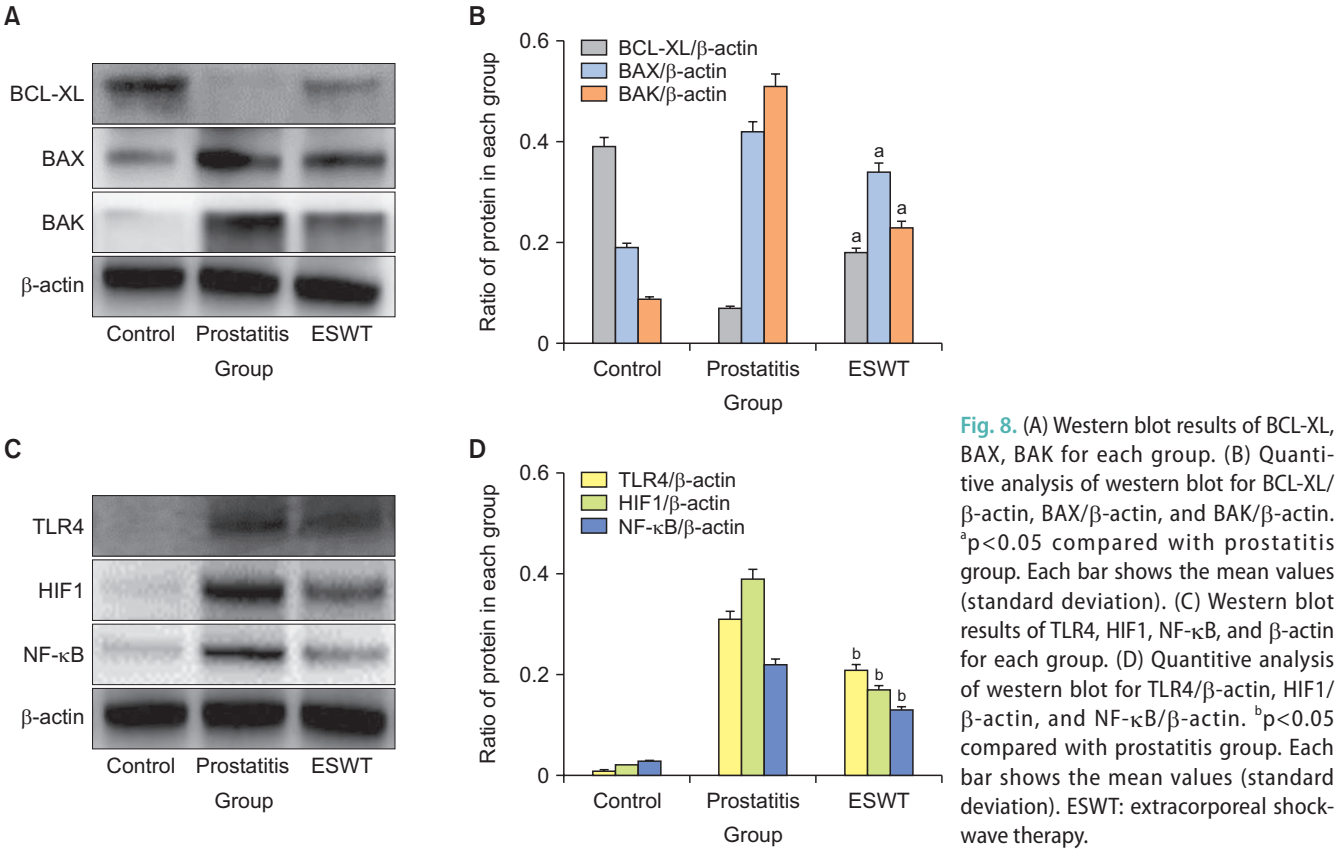

Fig. 8. (A) Western blot results of BCL-XL, BAX, BAK for each group. (B) Quantitive analysis of western blot for BCL-XL/β-actin, BAX/β-actin, and BAK/β-actin. [a]$p<0.05$ compared with prostatitis group. Each bar shows the mean values (standard deviation). (C) Western blot results of TLR4, HIF1, NF-κB, and β-actin for each group. (D) Quantitive analysis of western blot for TLR4/β-actin, HIF1/β-actin, and NF-κB/β-actin. [b]$p<0.05$ compared with prostatitis group. Each bar shows the mean values (standard deviation). ESWT: extracorporeal shockwave therapy.

with no definitive strategy to treat this condition.

Oral pharmacotherapy using antibiotics, anti-inflammatory agents (NSAIDs), alpha-adrenergic blockers, and neuromodulatory drugs [17] have been considered as first-line treatment for CP/CPPS which usually showed unsatisfactory outcomes [18]. Therefore, a multimodal pharmacologic and non-pharmacologic interventions are urgently warranted. Previously, ESWT a noninvasive treatment exhibited encouraging results in regenerative therapies [19]. It is now well accepted that the main symptom of CP/CPPS is pelvic discomfort in patients, which might be caused by inflammation [20] or due to neurogenic etiology [21]. Meanwhile, our previous studies revealed that ESWT improved potentially CP/CPPS by down-regulating inflammatory mediators [22], but the exact underlying mechanism was unclear. In the present study, we observed that ESWT exhibited attenuation of symptoms of CP/CPPS, and decreased NLRP3 expression by inhibiting TLR4-NFκB pathway at 0.2 mJ/mm^2 energy level. In addition, ESWT reduced apoptosis by down-regulating BAX/BAK pathway.

In a previous clinical study, patients with CPPS were subjected to ESWT at energy flux density of 0.25 mJ/mm^2 for 4 weeks [23]. The results revealed that the ESWT effectively attenuated CP/CPPS, as it was easy to apply and caused no undesirable side-effects in patients. In a pre-clinical study, researchers applied ESWT to male rats with symptoms of prostatitis, and demonstrated that 0.1 mJ/mm^2 ESWT attenuated symptoms of prostatitis in the short term [24]. In our previous study, we used 0.1 mJ/mm^2 ESWT on prostatitis rats and showed a positive result [22]. However, none of these studies provided a clear justification for the selection of 0.1 mJ/mm^2 or 0.2 mJ/mm^2 ESWT and which energy flux density could elicit an optimal result. To address this issue, in this experiment we first screened for an energy flux density for ESWT. We identified that at the energy flux density of 0.2 mJ/mm^2, EWST preferably stimulated VEGF expression and reduced COX-2 *in vitro*, and then improved regeneration and alleviated pain more effectively. Based on this result, we proceeded to an animal experiment using the energy flux density of 0.2 mJ/mm^2. We found that *in vivo* 0.2 mJ/mm^2 ESWT appeared to show a promising result to control symptoms of experimentally induced prostatitis in a rat model.

Previous reports suggested that NLRP3 inflammasomes were overexpressed, which promoted the maturation of IL-1β and IL-18 in the overall inflammatory process [5]. Yin et al [25] demonstrated that inflammation was evidently improved by inhibiting NLRP3 inflammasome activation *in vivo*. However, there were only a few studies attempted to link NLPR3 inflammasome as a mediator of inflammation specifically in prostatitis. Lu et al [26] administered rapamycin to a prostatitis rat model and showed decreased levels of IL-1β, IL-18, NLRP3, ASC and caspase-1 after this treatment. Another study also showed that CP/CPPS symptoms were improved by inhibiting NLRP3 inflammasome activation [27]. But none of these studies attempted to investigate the possible mechanism of action. In this experiment, we showed that the prostatitis symptoms were attenuated after NLRP3 reduction as well. Moreover, we proved that overexpressed NLRP3 inflammasome triggered apoptosis *in vivo* and the tissue injury was apparently decreased after ESWT mediated NLRP3 suppression.

An independent study found that NLRP3 inflammasome expression was impacted by NF-κB *in vitro* and the authors also proved the positive influence of TLR4 signaling on NLRP3 inflammasome activation [28]. But the correlation of TLR4 and NF-κB was not determined. In our previous study, we found TLR4/NF-κB pathway impacted apoptosis by regulating the expression of inflammatory factors. Furthermore, in this study, we determined that activation of BAX/BAK pathway was also decreased along with TLR4/NF-κB pathway. Cui et. al also proved inhibition of TLR4 decreased the level of Bax *in vitro* and *vivo* [29]. Moreover, TLR4/NF-κB pathway activation and NLRP3 activity were proved to be interrelated [30]. In our study, we illuminated that ESWT down-regulated TLR4/NF-κB pathway and successively NLRP3 inflammasomes was reduced as well. So, we concluded that the relationship between BAX/BAK pathway and NLRP3 inflammasomes was bonded by TLR4/NF-κB pathway. Activated TLR4/NF-κB pathway improved inflammation by decreasing NLRP3 inflammasomes and down-regulated apoptosis *via* inhibiting BAX/BAK pathway.

There are a few limitations in this study. First, we identified the optimal energy flux density of ESWT, but we didn't evaluate the optimal duration for the best results. We will evaluate this aspect in the next study by studying different treatment times. Second, we administered ESWT immediately after the induction of prostatitis in rats. So, these results may not

reflect an accurate outcome, because most patients with symptoms do not seek early intervention. Third, we proved TLR4 as the key protein linking NLPR3 inflammasome and BAX/BAK pathway by using changes in protein levels instead of blocking TLR4 expression using specific antagonists.

CONCLUSIONS

We show that ESWT improved prostate inflammation by reducing NLPR3 inflammasome and ameliorated apoptosis via inhibiting BAX/BAK pathway in a rat model. Our findings suggest that TLR4 might be the key protein linking NLPR3 inflammasome and BAX/BAK pathways. In conclusion, our findings strongly indicate that a role for ESWT in the treatment of CP/CPPS.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: MRR, WJB. Data curation: DS, JJP. Formal analysis: SK, YSC. Funding acquisition: BHP, HJJ. Investigation: SS, SC. Methodology: CWC, DUK. Project administration: JTC, SHP. Resources: SWK, MRR. Software: CWC, DUK. Supervision: SWK, MRR. Validation: SS, SC. Visualization: DS, JJP. Writing – original draft: WJB. Writing – review & editing: WJB.

Data Sharing Statement

The datasets supporting the conclusions of this article are included in this article.

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