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Intravesical Cannabidiol for Inflammation and Pain in Interstitial Cystitis/Bladder Pain Syndrome *via* TLR4/NF-κB and TRPV1 Modulation

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Purpose: This study explored the anti-inflammatory and analgesic mechanisms of intravesical cannabidiol (CBD) in cyclophosphamide (CYP)-induced interstitial cystitis/bladder pain syndrome (IC/BPS) rats.

Materials and Methods: Female Sprague-Dawley rats were divided into four groups of control, IC/BPS, IC/BPS+10 mg/kg CBD, and IC/BPS+100 mg/kg CBD (n=5/group). IC/BPS was induced by CYP injections, followed by intravesical CBD administration. Pain sensitivity and bladder function were assessed via Von Frey tests and cystometrograms. Histological, Western blot, and immunofluorescence analyses were performed on bladder tissues. SV-HUC1 cells were analyzed using western blot and scratch assays.

Results: CBD improved bladder function, reducing instability, prolonging intercontractile intervals, and enhancing detrusor contraction pressure. The CBD 100 mg/kg group showed greater pain relief in Von Frey tests compared with other groups. Histology revealed reduced inflammation, mast cell infiltration, and fibrosis in bladder tissues. CBD decreased TNF-α, COX2, IL-6, and TRPV1 levels and inhibited the TLR4/MyD88/pNF-κB pathway. In SV-HUC1 cells, CBD suppressed epithelial injury and downregulated TRPV1, TLR4, MyD88, p-NF-κB, and Bax/Bcl-xL, demonstrating anti-inflammatory and anti-apoptotic effects.

Conclusions: Intravesical CBD alleviates inflammation by inhibiting the TLR4/MyD88/pNF-κB pathway, reduces neuropathic pain via TRPV1 channels, and improves cell apoptosis and migration in CYP-induced IC/BPS model animals.

Keywords: Cannabidiol; Cystitis, interstitial; Neuroinflammatory diseases; TRPV cation channels

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INTRODUCTION

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a chronic and debilitating condition characterized by recurring pelvic pain, urinary urgency, urinary frequency, and discomfort, often accompanied by bladder inflammation [1]. In a large population-based, questionnaire-based prevalence study in the United States, 2.7% of women and 1.9% of men were diagnosed with IC/BPS [2]. While IC/BPS is a prevalent disorder, its etiology is unclear and its pathogenesis is complex [3]. Pathophysiological findings include chronic inflammation, dysfunction such as afferent hyperalgesia and upregulation of injury receptors, markedly elevated levels of C-reactive protein and pro-inflammatory cytokines, and increases in tumor necrosis factor-alpha (TNF-α) and nerve growth factor [4,5]. Traditional therapeutic approaches, including oral medications and invasive procedures, often yield limited relief and may carry adverse side effects [6]. Therefore, effective management and treatment for IC/BPS remain challenging.

Cannabidiol (CBD) is a non-psychoactive cannabinoid with a variety of biological activities and a wide range of benefits such as antioxidant, anti-inflammatory, and immunomodulatory effects. Research has demonstrated the therapeutic significance of CBD in neurological disorders, cardiomyopathy, diabetes, and other diseases [7,8].

Transient receptor potential vanilloid type 1 (TRPV1) is a nonselective transmembrane cation channel expressed by multimodal nociceptors. TRPV1 mediates thermal hyperalgesia and is closely related to pain transduction and detrusor function in IC/BPS [9].

In the present study, we investigated the potential therapeutic effects of CBD in IC/BPS. We conducted intravesical instillation of CBD in a rat model of IC/BPS and evaluated the effects on inflammation and bladder function and pain. We further analyzed its mechanism of action. Our study provides evidence of the potential effectiveness of CBD in the treatment of IC/BPS.

MATERIALS AND METHODS

1. Cell culture

SV-HUC-1 cells (ATCC) were cultured in F-12K medium (Gibco) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin solution (Gibco). Cells were maintained in a humidified incubator at 37 ℃ with 5% CO_2. Non-adherent cells were removed 24 h after seeding, and fresh medium was added. The medium was replaced every two days. When the cells reached approximately 80% to 90% confluence, they were subcultured using 0.25% trypsin-EDTA (Gibco). Detached cells were centrifuged at 300×*g* for 5 minutes, resuspended in fresh medium, and replated at an appropriate density for further expansion.

2. Cytotoxicity assay

CBD (KCA Labs), the MYD88 inhibitor ST2825 (MedChemExpress), and the TRPV1 inhibitor capsaicin (Sigma-Aldrich) were used in experiments. Cytotoxicity of the compounds was assessed to determine optimal treatment concentrations using the WST-1 assay kit (DoGenBio). SV-HUC-1 cells were seeded in 96-well plates (5,000 cells/well) in F-12K medium with 10% fetal bovine serum and 1% penicillin-streptomycin at 37 ℃ with 5% CO_2 for 24 hours. Stock solutions of CBD, ST2825, and capsaicin were prepared in DMSO and diluted to 0–100 μM in culture medium (<0.1% DMSO). After attachment, cells were treated with the compounds for 6 or 24 hours. Post-treatment, 10 μL of EZ-500 reagent was added, followed by a 2-hour incubation at 37 ℃. Absorbance was measured at 450 nm (reference: 650 nm). Experiments were performed in triplicate.

3. Cell processing

Inflammation was induced by treating SV-HUC-1 cells with TNF-α (Sigma-Aldrich) at a concentration of 20 ng/mL for 24 hours [10]. CBD was prepared as a 5 μM working solution in DMSO. ST2825 and capsaicin were dissolved in DMSO and diluted to final concentrations of 5 μM each. The cells were divided into the following treatment groups: Normal control, TNF-α group (20 ng/mL TNF-α), CBD group (TNF-α+5 μM CBD), ST2825 group (TNF-α+5 μM ST2825), and capsaicin group (TNF-α+5 μM capsaicin). Following treatment, cellular proteins were extracted and subjected to western blot analysis.

4. Scratch assay

SV-HUC-1 cells were seeded in 6-well plates and cultured until they reached approximately 90% confluence. A uniform scratch was created in the cell monolayer using a 200 μL pipette tip, and detached cells were removed by washing with phosphate-buffered sa-

line (PBS). The cells were then divided into two groups: the TNF-α group, treated with 20 ng/mL TNF-α, and the CBD group, treated with 20 ng/mL TNF-α and 5 μM CBD. Images of the scratched areas were obtained at 0, 12, and 24 hours using an inverted phase-contrast microscope to monitor wound closure.

5. Animals

Sprague-Dawley female rats (8 weeks old) were purchased from Orient Bio Co. Animals had free access to food and water and were housed under 12-hour light and dark cycles; the temperature of the feeding space was controlled at 22±2 ℃.

6. Induction of IC/BPS and drug therapy

Cyclophosphamide (CYP) was purchased from Sigma-Aldrich, and CBD was purchased from KCA Labs. IC/BPS was induced by intraperitoneal injection of 40 mg/kg of CYP every 3 days (days 0, 3, and 6) as described in previous studies [11]. Control animals received saline 5 mL/kg under the same experimental conditions. At days 7, 10, and 13 after injection, CBD was dissolved in saline (10 or 100 mg/kg) and was instilled intravesically under isoflurane respiratory anesthesia. The catheter was blocked and animals were held immobile for 15 minutes, followed by emptying the bladder, re-filling, and holding for another 15 minutes. The sham treatment group received 0.1 mL of saline intravesically under the same conditions.

7. Assessing nociception

To assess the nociceptive response in the bladder area after the final CBD or saline intravesical instillation, we used the Von Frey and Dynamic Plantar Aesthesiometers (DPA). Animals were placed in individual clear glass boxes with a wire mesh floor and acclimatized for at least 30 minutes before the start of the test. Von Frey filaments of force (1.65, 3.22, 4.08 and 6.01 g) were passed through the mesh floor and carefully stimulated for 1 to 2 seconds in different areas near the lower abdominal bladder to avoid desensitization. Sharp contraction of the abdomen, immediate licking, or grasping of the stimulated area of the filaments or jumping were considered positive responses [11]. The test was then carried out using the DPA, where the probe was automatically pushed out and stimulated the same area; the device automatically recorded the force at which a retraction response occurred. This was performed five times per animal, with stimuli at least 30 seconds apart, and the results were quantified and analyzed.

8. Assessment of bladder function

Continuous cytometric assessment was performed on treated rats placed in a supine position on top of the hotspot under respiratory anesthesia. The bladder was exposed by surgical incision of the midline of the abdomen; a catheter of PE-50 was inserted into the dome of the bladder and secured with sutures to prevent dislodgement [12]. The other end of the catheter was connected to a three-way plug also attached to a pressure transducer (ADInstruments) and a saline infusion pump. After the bladder was emptied, the infusion pump was operated at a rate of 0.15 mL/min, and the bladder pressure curve was recorded automatically by a computer for at least 30 minutes for each rat.

9. Assessment of histopathology and immunohistochemistry

Rat bladder tissue was removed after euthanasia of the animals and fixed in 4% formalin for 24 hours. After rinsing, bladder tissue was embedded in paraffin and sectioned at 5 or 10 μm thick. For all histological and immunohistochemical analyses, tissue sections were deparaffinized using xylene, followed by rehydration through graded ethanol solutions. To block nonspecific binding, the sections were incubated with 5% normal goat serum for 30 minutes at room temperature. After deparaffinization and rehydration, the sections were stained using a Masson trichrome staining kit (Abcam, ab150686) following the manufacturer's protocol. This method was used to assess collagen deposition and connective tissue changes.

For hematoxylin and eosin (H&E) staining, the sections were similarly deparaffinized, stained, and mounted using Poly-Mount (PolySciences Inc.) for structural analysis of the bladder [13].

For immunohistochemistry analysis, after the blocking step, the sections were incubated overnight at 4 ℃ with a primary antibody against Caspase-3 (1:500, Cell Signaling Technology, #9662S). After washing with PBS, the sections were treated with a rabbit-specific secondary antibody for 1 h at room temperature. Immunoreactivity was visualized using a DAB substrate kit (Vector Labs) following the manufacturer's instructions. The sections were counterstained with hema-

toxylin, dehydrated, and mounted.

For immunofluorescence analysis, after the blocking step, the sections were incubated with a primary antibody against TRPV1 (1:500, Abcam), followed by incubation with goat anti-rabbit Alexa Fluor 488-conjugated secondary antibody (1:500, Abcam). Nuclei were counterstained with DAPI (Abcam). Imaging was performed using a Zeiss LSM 900 Meta confocal microscope equipped with ZEN software (Carl Zeiss Microscopy) [14].

10. Western blotting analysis

Bladder tissues were homogenized and immersed in RIPA buffer (Cell Signaling Technology Inc.) on ice for 30 minutes. Following centrifugation at 12,000 rpm at 4 ℃ for 20 minutes, the supernatant was collected, and protein concentration was determined using a BCA protein assay kit. Equal amounts of protein (20 μg) were separated by electrophoresis on 10% Bolt Bis-Tris Plus gels (Thermo Scientific) and transferred to nitrocellulose membranes. The membranes were blocked for 1 h and then incubated with antibodies against TRPV1 (1:500, Abcam), TNF-α (1:1000, Santa Cruz), Cox2 (1:1,000, Cell Signaling Technology Inc.), IL-6 (1:2,000; Abcam), GAPDH (1:5,000, Abcam), TLR4 (1:1,000; Abcam), MyD88 (1:1,000; Abcam), p-NF-κB (1:1,000, Cell Signaling Technology Inc.), NF-κB (1:1,000, Cell Signaling Technology Inc.), Bax (1:1,000, Cell Signaling Technology Inc.), and Bcl-xL (1:1,000, Cell Signaling Technology Inc.). The membranes were incubated with horseradish peroxidase-conjugated horse anti-rabbit or anti-mouse IgG (GenDEPOT). Bands were visualized using enhanced chemiluminescence and a chemiluminescence imaging system. The density of each protein band was quantified using ImageJ software (National Institutes of Health).

11. Statistical analysis

Statistical analyses of data were Statistical analyses were performed using SPSS version 26.0 (IBM Corp.). Data are shown as mean±standard deviation. Multiple group comparisons were performed using analysis of variance (ANOVA) followed by the Tukey-Kramer test for post hoc comparisons. Data expressed as proportions were evaluated using the chi-square test. $p<0.05$ indicated statistical significance.

12. Ethics statement

All animal experiments in this study were approved by the Institutional Animal Care and Use Committee of the Catholic University of Korea, (IACUC approval No. CUMC-2023-0136-02).

RESULTS

1. Cytotoxicity assessment

CBD, ST2825, and capsaicin had no marked impact on SV-HUC-1 cell viability at concentrations ≤5 μM, with greater than 90% viable cells and no significant cytotoxicity observed (Fig. 1). Therefore, concentrations ≤5 μM were selected for subsequent cellular experiments.

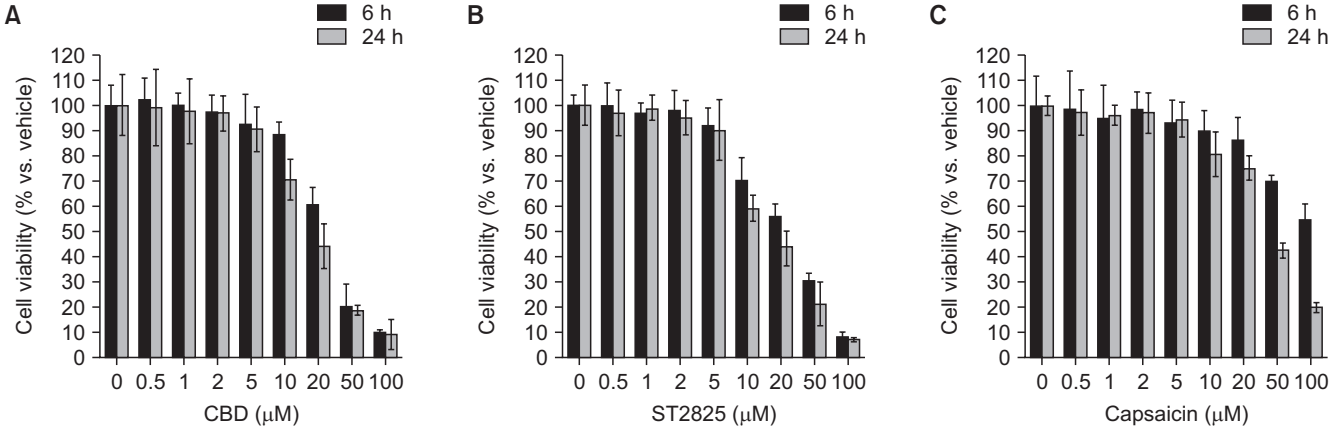

Fig. 1. Effect of cannabidiol (CBD), ST2825, and capsaicin on cell viability of SV-HUC-1 cells at 6 and 24 hours. Cell viability was assessed after treatment with different concentrations of (A) CBD, (B) ST2825, and (C) capsaicin for 6 hours (black bars) and 24 hours (gray bars). Concentrations ranged from 0 to 100 μM. Data are expressed as percentages relative to the vehicle control. Error bars represent the mean±standard deviation of at least three independent experiments.

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2. Pain assessment and cystometry results

Nociceptive response testing in rats showed a significant increase in response frequency and a marked decrease in the nociceptive threshold following CYP induction. After CBD treatment, the 100 mg/kg group exhibited a substantial reduction in pain withdrawal response frequency and a significant increase in pain threshold, indicating improved nociceptive responses. In contrast, the 10 mg/kg group showed no notable improvement in these measurements (Fig. 2A).

Continuous cystometric analysis was conducted after treatment, revealing that CYP-induced IC/BPS in rats significantly shortened bladder contraction intervals, increased basal bladder pressure, and reduced maximum bladder pressure, indicating decreased bladder compliance. In the CBD-treated groups, the 100 mg/kg dose notably increased maximum bladder pressure, while no significant changes were observed in the 10 mg/kg group. Both CBD-treated groups showed a reduction in basal bladder pressure and a prolongation of contraction intervals (Fig. 2B).

3. CBD inhibits TRPV1 expression in IC/BPS models induced in rat and SV-HUC-1 cells

To further investigate the mechanism by which CBD alleviates bladder pain, we performed immunodeficiency analysis of TRPV1 expression in rat bladder tissues. The results demonstrated a significant reduction in TRPV1 expression following CBD treatment (Fig. 3A). To confirm that CBD exerts its effects by reducing TRPV1 expression and promoting desensitization, we used the TRPV1 desensitization capsaicin. Western blot analysis revealed a reduction in TRPV1 protein expression following treatment with both CBD and capsaicin (Fig. 3B), consistent with previous findings [15-17].

4. CBD improves histological pathologies in CYP-induced IC/BPS: H&E and Masson staining results

Following treatment, H&E staining revealed signifi-

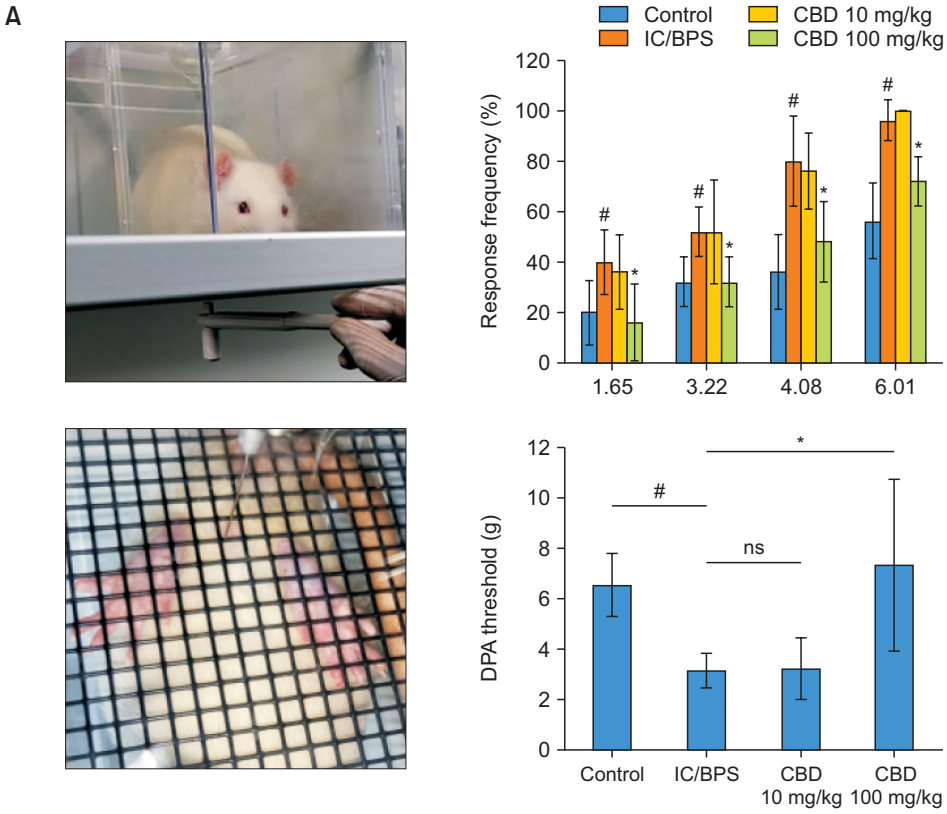

Fig. 2. (A) Representative images and quantitative results of the von Frey filament test for nociceptive responses and the DPA test for nociception in each experimental group. (B) Representative cystometry (CMG) images and bladder parameters, including maximum pressure, base pressure, and contraction interval, are shown. Data are expressed as mean±standard error (n=5). DPA: dynamic plantar aesthesiometer, IC/BPS: interstitial cystitis/bladder pain syndrome, CBD: cannabidiol. *p<0.05, **p<0.01 compared with the IC/BPS group; #p<0.05, ##p<0.01 compared with the control group.

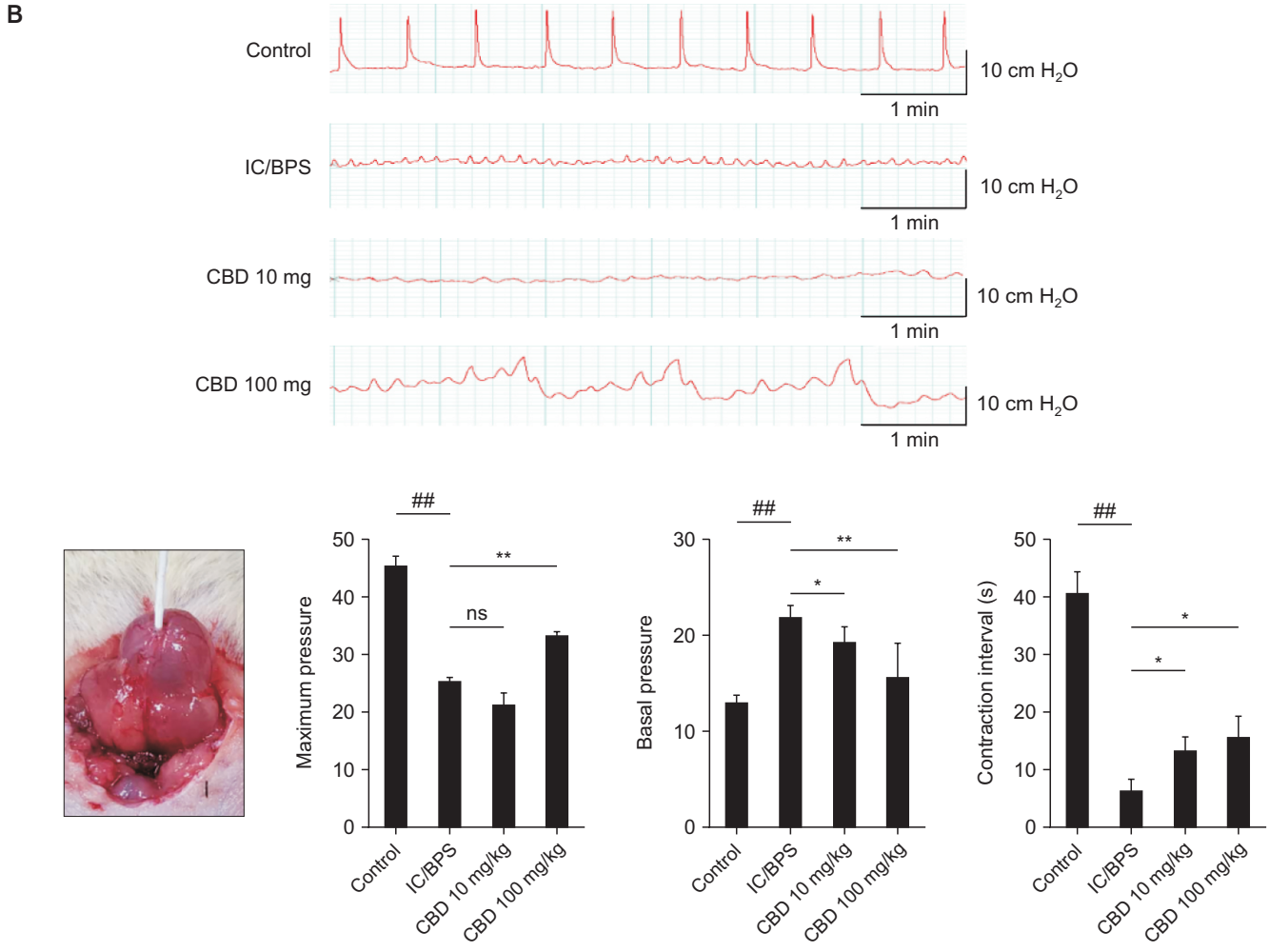

Fig. 2. Continued.

cant bladder mucosal congestion, edema, and epithelial ulceration in the IC/BPS group compared to the normal group. The CBD 10 mg/kg group showed slight improvement, while the 100 mg/kg group demonstrated near-complete repair of submucosal hemorrhage, edema, and epithelial ulceration (Fig. 4A). Masson's staining indicated increased collagen fiber deposition in the bladder submucosa and intermuscular bundles in the experimental group (Fig. 4B). Histological analysis confirmed CYP-induced bladder epithelial thinning, congestion, inflammatory infiltration, and fibrosis, all of which were significantly alleviated by CBD treatment. The bladder weight in the IC/BPS group (0.285±0.028 g) was significantly higher than that in the normal group (0.135±0.015 g). However, bladder weight was markedly reduced in the CBD 100 mg/kg treatment group (0.154±0.024 g). In contrast, there were no significant differences in body weight among the four groups. The bladder-to-body weight ratio in both the Normal and CBD 100 mg/kg treatment groups was significantly lower than that in the IC/BPS group ($p<0.05$). Furthermore, the muscle-to-collagen ratio in the IC/BPS group was significantly lower than that in the Normal and CBD treatment groups ($p<0.05$) (Fig. 4C).

5. CBD inhibits CYP-induced inflammation in rat bladder tissues and suppresses the TLR4/MyD88/NF-κB pathway in rat and SV-HUC-1 cells

Western blot analysis revealed that TNF-α, COX2, and IL-6 levels were significantly elevated in the IC/BPS group of rat bladder tissues. After CBD treatment, these inflammatory factors were markedly reduced, with a more pronounced decrease observed in the high-dose group (100 mg/kg) (Fig. 5).

Further analysis showed that CYP induction activat-

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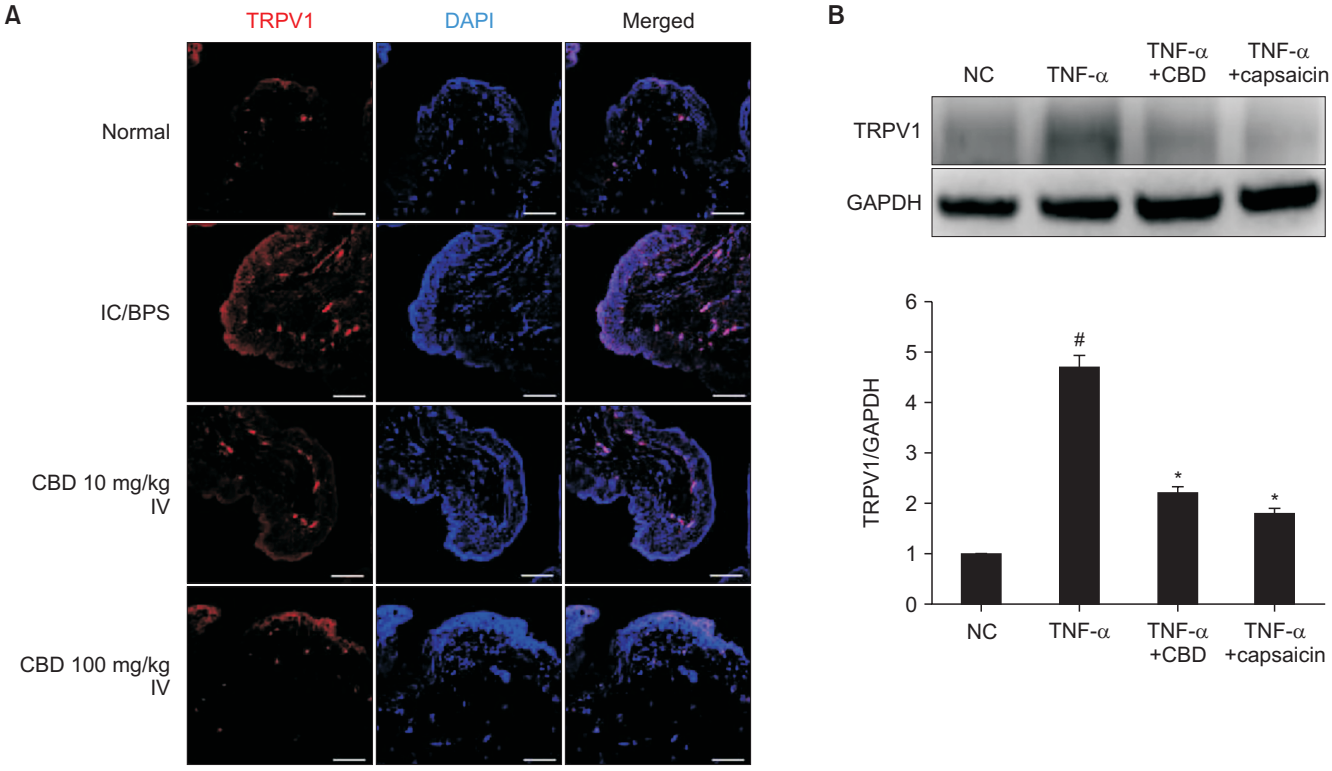

Fig. 3. (A) Representative images of TRPV1 immunofluorescence staining in bladder samples from the Control, IC/BPS, CBD 10 mg/kg, and CBD 100 mg/kg groups. TRPV1 expression is shown in red, with nuclei counterstained using DAPI in blue (scale bar=100 μm). (B) Western blot analysis of TRPV1 and GAPDH expression in bladder samples from the four groups, including representative blot images and quantitative analysis. IC/BPS: interstitial cystitis/bladder pain syndrome, CBD: cannabidiol, TNF-α: tumor necrosis factor-alpha, NC: normal control. Data are expressed as mean±standard error. *p<0.05 compared with the IC/BPS group; #p<0.05 compared with the Control group.

ed the TLR4/MyD88/NF-κB signaling pathway, while CBD treatment significantly reduced the expression of TLR4, MyD88, and phosphorylated NF-κB, indicating an inhibitory effect on this signaling pathway (Fig. 6A). To further explore the mechanism, the biological effects of CBD and ST 2825 (a MyD88 inhibitor) were evaluated in SV-HUC-1 cells. CBD suppressed TNF-α-driven expression of TLR4, MyD88, and phosphorylated NF-κB (pNF-κB). Similarly, treatment with ST 2825 significantly reduced MyD88 and pNF-κB levels (Fig. 6B).

6. CBD promotes cell survival and repair in IC/BPS by regulating apoptosis and enhancing migration in rat bladder and SV-HUC-1 cells

Immunohistochemistry analysis revealed strong Caspase-3 expression in the IC/BPS group, indicating increased apoptosis. However, the 100 mg/kg CBD treatment group exhibited a significant reduction in caspase-3 expression (Fig. 7A).

Western blot analysis showed that TNF-α treatment increased Bax expression and decreased Bcl-xL expression in SV-HUC-1 cells, resulting in a higher Bax/Bcl-xL ratio. Co-treatment with 5 μM CBD significantly reduced the Bax/Bcl-xL ratio, indicating that CBD regulated apoptosis by balancing pro-apoptotic and anti-apoptotic proteins (Fig. 7B).

Scratch assay results showed that TNF-α significantly impaired cell migration and wound closure in SV-HUC-1 cells, while treatment with 5 μM CBD promoted cell migration and accelerated wound healing, particularly at 24 hours (Fig. 7C).

DISCUSSION

IC/BPS is characterized by chronic pelvic pain, frequent urination, and bladder inflammation. Current treatments often provide limited relief, necessitating the exploration of alternative therapies. CBD is of interest for its anti-inflammatory and analgesic properties, and we hypothesized that it could be of benefit to

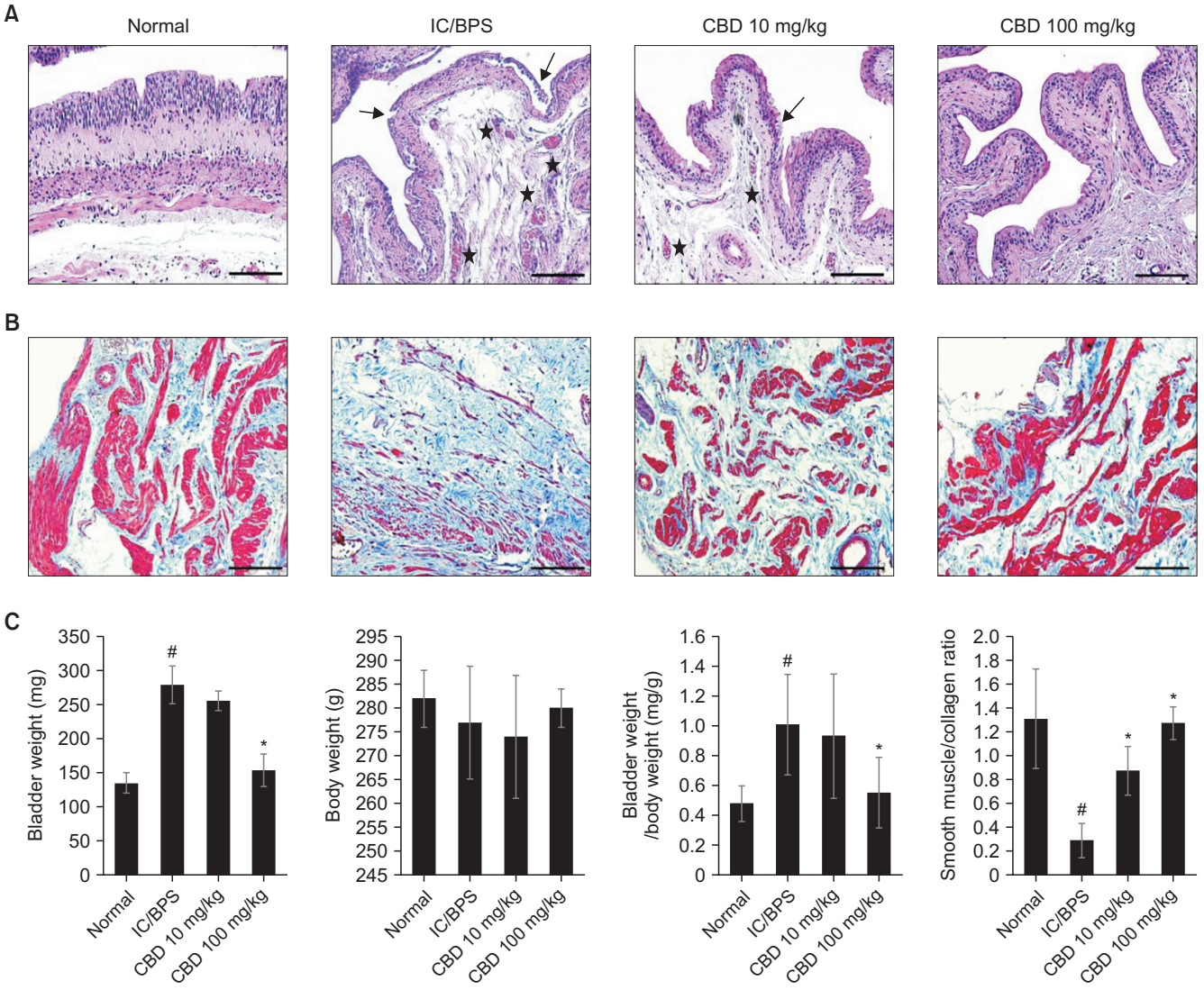

Fig. 4. (A) Representative images of bladder tissue stained with hematoxylin and eosin (H&E), showing the Normal control group, IC/BPS group, CBD 10 mg/kg treatment group, and CBD 100 mg/kg treatment group. The IC/BPS group exhibited severe urothelial ulceration (arrows), marked edema, and lamina propria congestion (stars) compared to the Normal control group. In the CBD 10 mg/kg group, mild urothelial ulceration (arrows), mild lamina propria congestion (stars), and slight edema were observed. The CBD 100 mg/kg group showed nearly normal urothelium, submucosal lamina propria, and muscularis propria (scale bar=100 μm); (B) Masson's trichrome staining of bladder tissue revealed reduced fibrosis in the CBD-treated groups (scale bar=200 μm); (C) Bladder weight, body weight, bladder-to-body weight ratio, and muscle-to-collagen ratio in each group. Data are expressed as mean±standard deviation. IC/BPS: interstitial cystitis/bladder pain syndrome, CBD: cannabidiol. *p<0.05 compared with the IC/BPS group; #p<0.05 compared with the control group.

patients with IC/BPS. One promising line of research is the use of CBD via intravesical therapy, which involves infusing the drug directly into the bladder via a catheter. This local delivery system has the advantage of directly targeting the affected area, potentially minimizing systemic side effects while maximizing efficacy.

Previous studies have found that the mechanism of CBD analgesia and inhibition of bladder overactivity may be through the involvement of TRPV1 channels [18,19]. The first identified role of TRPV1 in the urinary tract was in pain perception [20]. However, there is also an important role for TRPV1 in the regulation of reflex contraction frequency in the bladder [13]. Some studies have found success in the use of capsaicin and other receptors to desensitize TRPV1 for the treatment of painful bladder and neurogenic or non-neurogenic overactive bladder [14,21,22]. In DRG neurons treated with 10 and 50 μM/L CBD, calcium currents were reduced in a dose-dependent manner and physiologically

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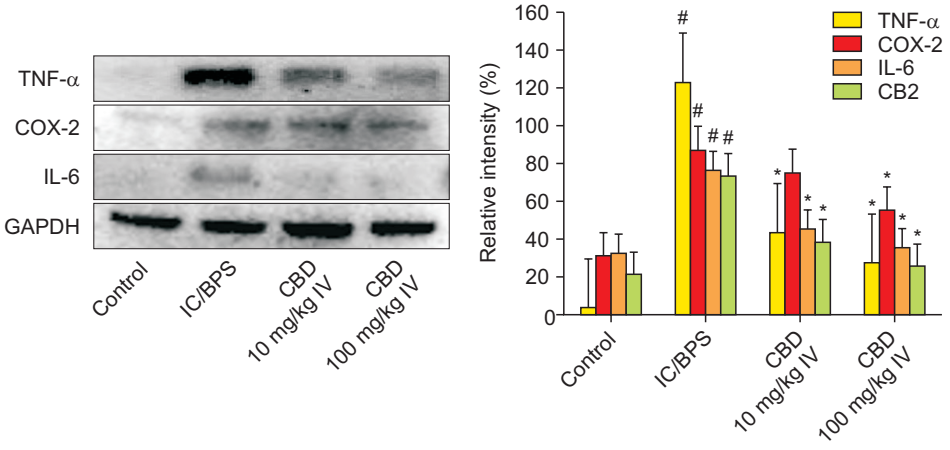

Fig. 5. Western blot analysis and densitometric quantification of TNF-α, COX-2, and IL-6 expression in rat bladder tissues from the control, IC/BPS, CBD 10 mg/kg, and CBD 100 mg/kg groups. Expression levels were normalized to GAPDH. Data are expressed as mean±standard deviation. TNF-α: tumor necrosis factor-alpha, IL: interleukin, IC/BPS: interstitial cystitis/bladder pain syndrome, CBD: cannabidiol. *p<0.05 compared with the IC/BPS group; #p<0.05 compared with the control group.

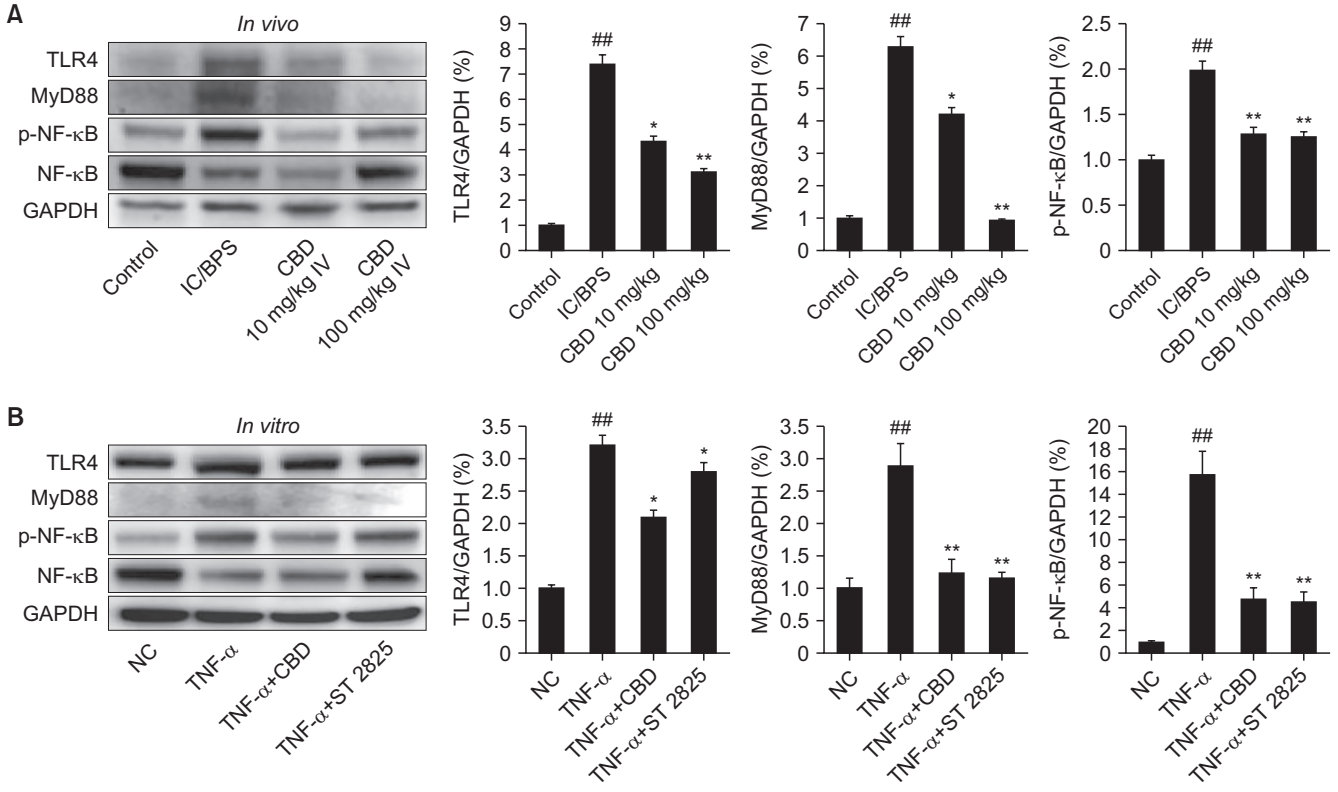

Fig. 6. (A) Western blot analysis and densitometric quantification of TLR4, MyD88, and phosphorylated NF-κB (p-NF-κB) expression in rat bladder tissues from the Control, IC/BPS, CBD 10 mg/kg, and CBD 100 mg/kg groups. Data are expressed as mean±standard deviation (SD). *p<0.05, **p<0.01 compared with the IC/BPS group; ##p<0.01 compared with the control group. (B) Western blot analysis and densitometric quantification of TLR4, MyD88, and phosphorylated NF-κB (p-NF-κB) expression in SV-HUC-1 cells across the Control, TNF-α, TNF-α+CBD, and TNF-α+ST2825 treatment groups. Cells were treated with TNF-α alone or in combination with CBD or ST2825 for 24 hours. Data are expressed as mean±SD. *p<0.05, **p<0.01 compared with the TNF-α group; ##p<0.01 compared with the control group. IC/BPS: interstitial cystitis/bladder pain syndrome, CBD: cannabidiol, TNF-α: tumor necrosis factor-alpha, NC: normal control.

inhibited or reduced the sensitivity of neuronal TRPV1 signaling by inhibiting the adenylate cyclase-cAMP pathway [9]. The desensitization of TRPV1 by CBD was similar to that of capsaicin-stimulated desensitization but was significantly greater than that by capsaicin stimulation [23,24]. Our data suggest that CBD reduces pain behavior in the rat model and causes improvements in bladder function, with significant normalization of intercontractile intervals and bladder compliance compared with effects in the IC/BPS group, suggesting a restorative effect on bladder physiology. TRPV1 expression was significantly reduced in the

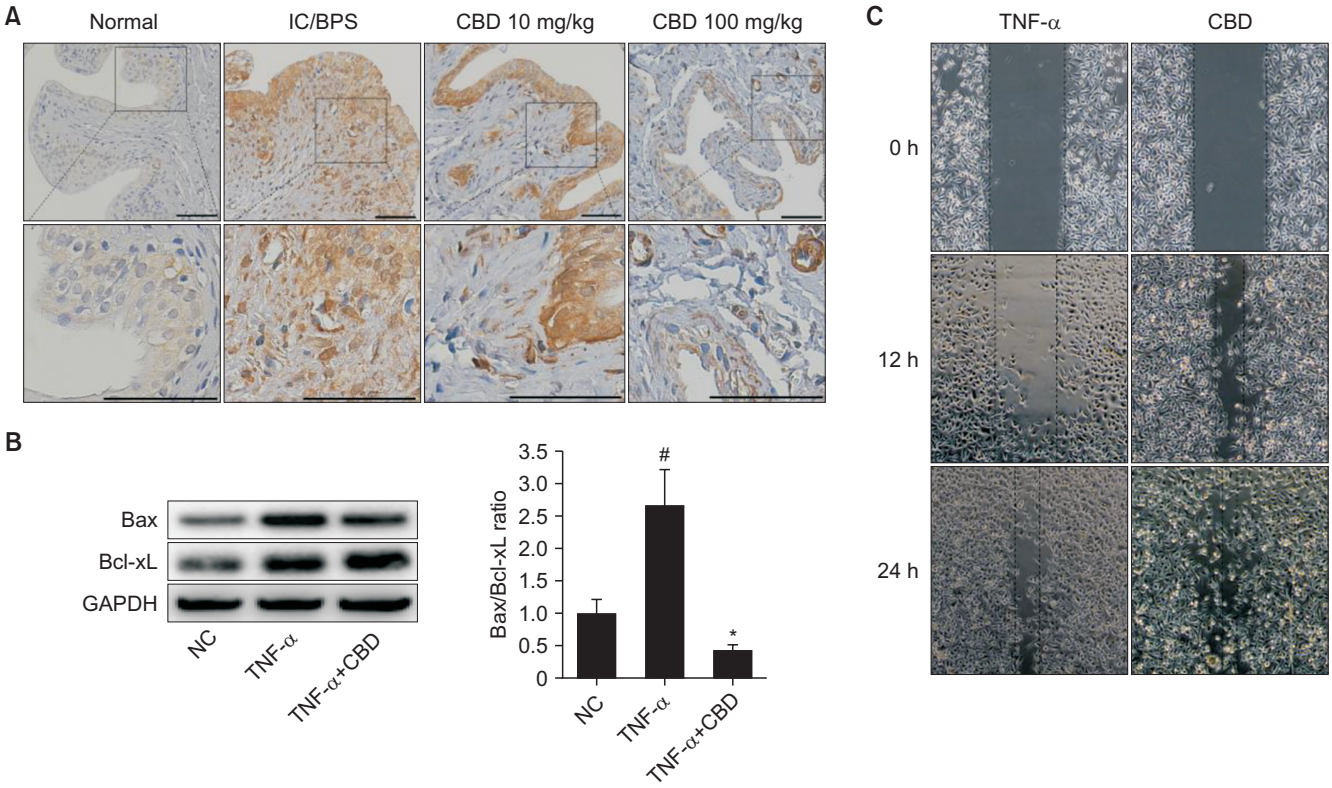

Fig. 7. (A) Immunohistochemistry (IH) analysis of caspase-3 expression in different groups: normal control, IC/BPS, and CBD treatment groups (10 mg/kg and 100 mg/kg). Strong caspase-3 expression was observed in the IC/BPS group, while the 100 mg/kg CBD treatment group showed a significant reduction in expression (scale bar=100 μm). (B) Western blot analysis of Bax and Bcl-xL expression in SV-HUC-1 cells across the control, TNF-α, and TNF-α+CBD treatment groups, with GAPDH used as a loading control to confirm equal protein loading. Quantitative analysis of the Bax/Bcl-xL ratio is shown as a bar graph. Cells were treated with TNF-α alone or in combination with CBD for 24 hours. (C) Scratch assay on SV-HUC-1 cells with TNFα and CBD treatment groups. Images were taken at 0, 12, and 24 hours to assess cell migration and wound closure. Data are expressed as mean±standard deviation. IC/BPS: interstitial cystitis/bladder pain syndrome, CBD: cannabidiol, TNF-α: tumor necrosis factor-alpha, NC: normal control. *p<0.05 compared with the TNF-α group; #p<0.01 compared with the control group.

CBD-treated group. These findings are consistent with studies on the role of CBD in regulating pain and overactive bladder disorders via TRPV1 [15].

Previous studies have shown that the induction of IC/BPS by CYP is mediated through the NF-κB signaling pathway [16,17]. One study found that CBD reduced microglia activation induced by the TLR4 agonist lipopolysaccharide (LPS), as well as levels of pNF-κB p65, TNFα, and NADPH synthesis [25]. Scott et al [26] demonstrated that CBD pretreatment prior to LPS treatment in the human acute monocytic leukemia cell line THP-1 appeared to affect the TLR4/MyD88 pathway. In the RAW264.7 murine macrophage cell line, CBD effectively inhibited NF-κB activation and reduced the mRNA levels of IL-1β, TNFα, and MCP-1 [25]. Our findings suggest that CBD effectively inhibits the TLR4/MyD88/NF-κB signaling pathway in the CYP-induced rat IC/BPS model. The observed reduction in the levels of pro-inflammatory cytokines (*e.g.*, TNF-α, IL-6, and IL-1β) supports the anti-inflammatory properties of CBD. This is consistent with previous studies that demonstrated the potential of CBD to reduce inflammation in various inflammatory disease models.

Studies suggest that CBD may interact with various intracellular molecular targets related to antioxidant, anti-inflammatory, and anti-apoptotic pathways to exert its biological effects [27]. One report demonstrated that LPS-induced mouse kidney injury involved CBD down-regulation of the expression of GRP78, CHOP, and caspase-3; decrease of the expression of Bcl-2 and NRF2; and enhancement of caspase-9 expression, indicating that CBD inhibits apoptosis development via mitochondrial stress [28]. In the current study, CBD downregulated the expression of caspase-3 and reduced the Bax/Bcl-xL ratio in CYP-induced IC/BPS in rats. This protective mechanism involves down-regulation of

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endoplasmic reticulum stress-induced apoptotic effectors and upregulation of the anti-apoptotic protein Bcl-2 [28]. Furthermore, CBD inhibits apoptosis by enhancing mitochondrial function. Our research also revealed that CBD promotes the migration and proliferation of urothelial cells following TNF-α stimulation. The proliferative effects of CBD are mediated through the activation of MEK1, which in turn activates downstream molecules, including ERK1/2 [29].

Although our study provides valuable insights into the potential benefits of CBD for IC/BPS, several limitations should be considered. One key limitation is that we did not determine the optimal therapeutic dose of CBD, as our evaluation was limited to two concentrations (10 mg/kg and 100 mg/kg). Future studies will need to explore a broader range of doses to identify the most effective concentration. Additionally, while we employed TRPV1 and MyD88 inhibitors to investigate the signaling pathways involved in CBD's effects, the reliability of this approach requires further validation. To strengthen these findings, additional forward and reverse experiments will be necessary in subsequent studies. Moreover, previous research has demonstrated that the endocannabinoid system exerts immune and anti-inflammatory effects through CB2 receptor activation. Given that CBD has a relatively weak affinity for CB2 receptors and may instead modulate the endocannabinoid system indirectly, future studies should compare its effects with those of CB2 receptor agonists to gain a deeper understanding of its mechanism of action.

In summary, this study demonstrates that intravesical CBD alleviates bladder inflammation, pain behavior, and functional abnormalities in a CYP-induced IC/BPS model, potentially through multiple interrelated mechanisms. These include TRPV1 desensitization, inhibition of the TLR4/MyD88/NF-κB signaling pathway, and suppression of endoplasmic reticulum stress-induced apoptosis. The observed urothelial regeneration via the MEK/ERK pathway further supports CBD's therapeutic potential. These mechanistic insights not only reinforce the anti-inflammatory, anti-apoptotic, and analgesic effects of CBD, but also provide a rationale for its localized intravesical application, which may minimize systemic exposure. Although our findings are promising, further studies are needed to determine the optimal therapeutic dose, validate pathway-specific effects, and evaluate safety and pharmacokinetics in large-animal models or early-phase clinical trials to support clinical translation in IC/BPS treatment.

CONCLUSIONS

Intravesical CBD alleviates inflammation by inhibiting the TLR4/MyD88/pNF-κB signaling pathway, reduces neuropathic pain through TRPV1 channel activation, and enhances cell survival by regulating apoptosis and promoting cell migration in CYP-induced IC/BPS rats.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: WJB, SWK. Methodology: JJP. Software: SK. Validation: HJL. Formal analysis: GQZ. Resources: GQZ. Data curation: KHJ. Visualization: WJT. Supervision: ZCX. Project administration: SK, HJL. Funding acquisition: SWK. Writing – original draft: JJP, SHP. Writing – review & editing: RLG. All authors have read and agreed to the published version of the manuscript.

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