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ORIGINAL RESEARCH

Adenosine A_{2A} Receptor Agonist Polydeoxyribonucleotide Alleviates Interstitial Cystitis-Induced Voiding Dysfunction by Suppressing Inflammation and Apoptosis in Rats

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Background: Interstitial cystitis (IC) is a chronic disorder that indicates bladder-related pain or discomfort. Patients with IC often experience urination problems, such as urinary frequency and urgency, along with pain or discomfort in the bladder area. Therefore, new treatments based on IC etiology are needed. Polydeoxyribonucleotide (PDRN) is a biologic agonist of the adenosine A_{2A} receptor, and PDRN has anti-inflammatory effect and inhibits apoptosis. In the current study, the effect of PDRN on cyclophosphamide-induced IC animal model was investigated using rats.

Methodology: To induce the IC animal model, 75 mg/kg of cyclophosphamide was injected intraperitoneally once every 3 days for 10 days. The rats in the PDRN-treated groups were intraperitoneally injected with 0.5 mL physiological saline containing 8 mg/kg PDRN, once a day for 10 days after IC induction.

Results: Induction of IC by cyclophosphamide injection caused voiding dysfunction, bladder edema, and histological damage. Cyclophosphamide injection increased secretion of pro-inflammatory cytokines and enhanced apoptosis. In contrast, PDRN treatment alleviated voiding dysfunction, bladder edema, and histological damage. Secretion of pro-inflammatory cytokines and expressions of apoptotic factors were suppressed by PDRN treatment. These changes indicate that treatment with PDRN improves voiding function by ultimately promoting the repair of damaged bladder tissue.

Conclusion: The conclusion of this experiment suggests the possibility that PDRN could be used as an effective therapeutic agent for IC.

Keywords: interstitial cystitis, adenosine A_{2A} receptor, polydeoxyribonucleotide, voiding function, inflammation, apoptosis

Introduction

Interstitial cystitis (IC) is a chronic disorder that indicates bladder-related pain or discomfort. Patients with IC often experience urination problems, such as urinary frequency and urgency, along with pain or discomfort in the bladder area.[1] IC is an important disease that degrades quality of life, but nevertheless, there is no clear treatment option because the clinical phenotypes of IC are very diverse and there is insufficient information about the underlying mechanisms of IC.[2] Therefore, a key priority in IC research is exploring the molecular mechanisms of this disease in order to develop new, effective, and safe treatments.

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The underlying molecular processes in the pathogenesis of IC involve complex interactions between inflammation and apoptosis.[3,4] Pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, exacerbate the process of diseases, and play a particularly important role in the progression of IC.[5,6] During IC progress, various damaging factors promote inflammation of bladder, increasing bladder tissue damage and contributing to the apoptosis process.[7]

Mitogen-activated protein kinases (MAPK), composed of extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38, involved in the development of many diseases through activation of nuclear factor-κB (NF-κB).[8] MAPK is associated with inhibition of cell growth, inflammation, and with mediating apoptosis in different cell types.[9] MAPK and NF-κB are phosphorylated and then activated by many stimuli such as cytokines and cell stress.[10] Activation of MAPK/NF-κB can promote the expression of various pro-inflammatory cytokines, thereby regulating inflammation and apoptosis.[10,11] Enhancement of MAPK/NF-κB activity has been demonstrated in urothelial cells of IC,[12] and increased phosphorylation of MAPK cascade has been found in the bladder of IC patients.[13] Therefore, MAPK/NF-κB signaling pathway serves as a key component in the progress of IC.[14–16]

Adenosine and its receptors are an essential neuromodulator that plays an important role in various pathophysiological conditions. In particular, adenosine receptors are located in the epithelium of bladder and may regulate normal bladder function.[17] There are four subtypes of adenosine receptors such as A_1, A_{2A}, A_{2B}, and A_3. Among them, the adenosine A_{2A} receptor is involved in the inflammatory reaction and, when activated, suppresses the secretion of pro-inflammatory cytokines.[18] In addition, evidences suggest that A_{2A} receptor is involved in the regulation of the voiding reflex and neuropathic pain.[17,19]

Polydeoxyribonucleotide (PDRN), adenosine A_{2A} receptor agonist, has suppressing effect on the production of pro-inflammatory cytokines. PDRN inhibits inflammatory cytokines production and apoptosis in various diseases such as gastric ulcers, acute lung injury, and ischemic colitis.[20–22] However, despite the excellent pharmacological efficacy of anti-inflammatory and anti-apoptotic action, few studies have evaluated the effectiveness of PDRN in urological diseases. If PDRN can inactivate the signaling pathway of MAPK/NF-κB, PDRN may have a positive effect on the treatment process of IC.

Therefore, we evaluated the effect of the MAPK/NF-κB signaling on voiding problems in the IC animal model. Moreover, the effect of A_{2A} receptor agonist PDRN on the MAPK/NF-κB signaling pathway associated inflammation, apoptosis, and voiding dysfunction was investigated using rats.

Materials and Methods

Animals and Classification

Twelve-week-old female Sprague-Dawley rats (250 ± 10 g, n = 40) were purchased from a commercial breeder (Orient Co., Sungnam, Korea) for this research. The rats were randomly classified into four groups: control group, IC-induced group, IC-induced and PDRN-treated group, and IC-induced and PDRN with 7-dimethyl-1-propargylxanthine (DMPX) treated group 100 (n = 10 each group).

This experimental procedure was approved by the Institutional Animal Care and Use Committee of Kyung Hee University and received the following approval number (KHUASP[SE]-18-096). The experimental process was conducted in good faith in accordance with the animal care guidelines from the National Institutes of Health and the Korean Institute of Medical Sciences.

Induction of IC and Treatments

IC model was made by injection of cyclophosphamide in the same method as described below.[23] To induce IC rats, the rats received intraperitoneal injection with 75 mg/kg cyclophosphamide (Sigma Chemical Co., St. Louis, MO, USA) every 3 days for 10 days (total of 3 injections). The rats in the control group received an intraperitoneal injection with the same volume of physiological saline on the same schedule. The rats in the PDRN treated groups were intraperitoneally injected with 0.5 mL physiological saline containing 8 mg/kg PDRN (Rejuvenex®, PharmaResearch Products, Pangyo, Korea), once daily for 10 days after IC induction (Figure 1). We selected PDRN concentration that was found to be most effective through previous studies[20,21] and preliminary experimental result. In order to confirm that the action of PDRN occurs through the adenosine A_{2A} receptor, 8 mg/kg DMPX (Sigma Aldrich Co.) was co-treated with PDRN.

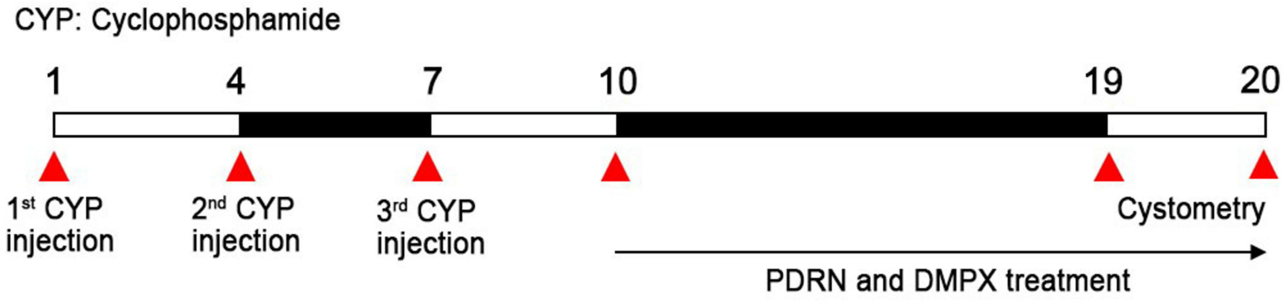

Figure 1 Experimental schedule.

Cystometry Analysis

In the cystometry analysis, contraction pressure and contraction time were determined 19 to 20 days after IC induction in the same method as described below.[23,24] After anesthesia with intraperitoneal injection with Zoletil 50® (10 mg/kg; Vibac Laboratories, Carros, France), a transperitoneal incision was made and a polyethylene catheter (PE50) was inserted into the bladder. A syringe pump (Havard Apparatus, Holliston, MA, USA) and a pressure transducer (Havard Apparatus) were connected via a three-way stopcock to inject saline into the bladder and simultaneously record the intra-bladder pressure. After emptying the bladder, 0.5 mL saline was injected, and a pressure-flow study was conducted. LabScribe (iWork System Inc., Dover, NH, USA) was used to measure the contraction pressure and contraction time of the bladder.

Tissue Preparation

Tissue preparation was conducted in the same method as described below.[24] The rats were anesthetized with Zoletil 50® (10 mg/kg; Vibac Laboratories) 20 days after first administration of cyclophosphamide. The extracted bladder tissue was measured by wet-weight and the ratio to body weight was calculated. After the bladder tissue was harvested, the bladder tissue was sufficiently fixed in 4% paraformaldehyde, dehydrated with ethanol series (70%, 80%, 90%, 95%, and 100%), treated with xylene, and embedded in paraffin. Paraffin-embedded bladder tissue was cut to 5 μm thick using a microtome (Thermo Fisher Scientific, Waltham, MA, USA) and mounted on coated slides (6 sections per bladder tissue). Slides with bladder tissue were dried overnight at 37°C on a hot plate.

Hematoxylin and Eosin (H&E) Staining

To observe histological changes in the bladder tissues, H&E staining was conducted in the same method as described below.[24,25] The slides were soaked in Harris hematoxylin (Young-Dong Diagnostics, Yongin, Korea) for 10 seconds and then washed with clean water. Then, the slides were dipped in eosin (Sigma Chemical Co.) for 5 seconds and washed again with distilled water. After drying the stained slides, the slides were dehydrated by dipping with ethanol and xylene, and then sealed by Permount® (Thermo Fisher Scientific).

The inflammatory score was calculated to indicate tissue damage in the same method as described below.[26] Images of H&E stained slides were taken with a Leica Application Suite X Computer-Assisted Image Analysis System (Leica Microsystems, Wetzlar, Germany) attached to a light microscope (Leica DMi8, Leica Microsystems). The degree of bladder inflammation was analyzed using a 6-point scale in a double-blind manner of pathologist. Criteria for histological evaluation of bladder inflammation are presented in Table 1.

Pro-Inflammatory Cytokines and cAMP Concentrations

For the ELISA analysis in bladder tissue, concentrations of TNF-α, IL-1β, and cAMP were measured by reacting with enzyme immunoassay kit (Abcam, Cambridge, UK) according to the manufacturer's manual. For TNF-α and IL-1β measurement in the bladder tissue, standard or lysed sample was added to each well, and plates were incubated at room temperature for 2.5 hours. The prepared TNF-α and IL-1β biotin antibodies were added to each well and incubated at room temperature for 1 hour. Then, HRP-streptavidin solution was added and incubated at room temperature for 45 minutes. TMB one-step development solution was reacted at room temperature for 30 minutes in the dark on a shaker. Immediately, stop solution was added to each well and calculated at 450 nm wavelength with Multiskan Go Microplate Readers (Thermo Fisher Scientific). For cAMP calculation, prepared standard and

Table 1 Histological Score

Score	Histological Characteristics
0	Morphologically unremarkable with no or very minimal inflammation or epithelial changes.
1	Minimal inflammatory infiltrate composed of occasional neutrophils or lymphocytes within the lamina propria in the absence of inflammation in the muscularis propria, or significant edema, hemorrhage or urothelial changes.
2	Minimal to mild inflammatory infiltrate within the lamina propria with scattered neutrophils or lymphocytes, accompanied by mild edema or hemorrhage, but in the absence of inflammation in the muscularis propria or significant urothelial changes.
3	Mild or mild to moderate inflammatory infiltrate in the lamina propria and focal extension of the inflammation into the muscularis propria.
4	Moderate inflammation with scattered to frequent neutrophils and lymphocytes in both the lamina propria and muscularis propria.
5	Severe inflammation in the lamina propria and muscularis propria in association with other significant findings, such as urothelial ulceration, severe edema, hemorrhage and fibrin deposition.

Table 2 Primary and Secondary Antibodies in Western Blot

Classification	Items	Source	Titer	Company
Primary antibody	NF-κB	Anti-rabbit	1:1000	Abcam, Cambridge, UK
	IκB-α, p-IκB-α, CREB, p-CREB, PKA, p-PKA	Anti-rabbit	1:1000	Santa Cruz Biotechnology, CA, USA
	ERK, p-ERK, JNK, p-JNK, p38, p-p38	Anti-rabbit	1:2000	Cell Signaling Technology, Danvers, USA
	Bax, Bcl-2, β-actin	Anti-mouse	1:1000	Santa Cruz Biotechnology, CA, USA
Secondary antibody	HRP-conjugated IgG	Mouse Rabbit	1:2000	Vector Laboratories, CA, USA

Abbreviation: p, phosphorylation.

diluted samples were added to wells. Then, prepared alkaline phosphatase-conjugate was added to wells. Subsequently, cAMP complete antibody was reacted at room temperature on a shaker for 2 hours (500 rpm). pNpp substrate solution was added to wells and incubated at room temperature for 1 hour. Then, stop solution was added and read at 405 nm wavelength with Multiskan Go Microplate Readers (Thermo Fisher Scientific).

Western Blot Analysis

Western blot analysis was conducted in the same method as described below.[21] The bladder tissue was homogenized on chilled RIPA buffer (Cell Signaling Technology, Inc., Danvers, MS, USA) with 1 mM PMSF (Sigma Aldrich), and then centrifuged at 14,000 rpm for 30 minutes at 4°C. Protein contents were measured using a μ-drop reader (Thermo Fisher Scientific), and 30 μg protein was separated on SDS-PAGE gels and transferred to a nitrocellulose membrane. The primary and secondary antibodies used are as shown in Table 2.

After the primary (NF-κB, IκB-α, phosphorylated (p)-IκB-α, ERK, p-ERK, JNK, p-JNK, p38, p-p38, CREB, p-CREB, PKA, p-PKA, Bax, Bcl-2, and β-actin) and secondary antibodies (mouse and rabbit horseradish peroxidase (HRP)-conjugated IgG) reactions, the blot membranes were detected by an enhanced chemiluminescence (ECL) detection kit (Bio-Rad, Hercules, CA, USA).

To compare the relative expressions of target proteins, the detected bands were calculated with Image-Pro® plus computer-assisted image analysis system (Media Cyberbetics Inc., Silver Spring, MD, USA). For the relative quantification of the band, the result of the control group was set to 1.00.

TUNEL Assay

TUNEL assay was performed using a In Situ Cell Death Detection Kit® (Roche, Mannheim, Germany) according to the manufacturer's protocol in the same method as described below.[27,28] The slides containing bladder tissue were deparaffinized using xylene, rehydrated with graded ethanol, and then rehydrated by running water for 5 minutes. The slides were boiled 10 mM sodium citrate buffer at 95°C for 2 minutes. Subsequently, slides were cooled for 30 minutes at room temperature. The slides were washed with phosphate buffered saline for 5 minutes to three times. Slides were incubated with label solution and enzyme solution mixture at 37°C for 1 hour. After wash

out, slides were mounted on a coverslip using Vectashield® containing DAPI (Vector Laboratories, CA, USA). Fluorescence images were acquired by the Leica DMi8 microscope (Leica Microsystems) at excitation wavelength in the range of 450–500 nm and detection in the range of 515–565 nm (Leica Microsystems).

To identify the TUNEL-positive cells, five fields of view in the bladder mucosa were randomly selected from each sample, and more than 100 cells per field were counted under × 400 magnification. The percentage of TUNEL-positive cells was calculated by the following formula: number of TUNEL-positive cells/number of total cells × 100 (%).

Data Analysis

Multiple comparisons were conducted by SPSS software (version 23.0, IBM Corporation, Armonk, NY, USA). Statistical analysis between the groups was conducted using one-way ANOVA followed by Duncan's post-test. The data were presented as the mean ± standard error of the mean, and $P < 0.05$ was found to be statistically significant.

Results

Voiding Function

The analysis of voiding function from cystometry are presented in Figure 2A–D. Induction of IC by cyclophosphamide injection increased contraction pressure and contraction time while reducing inter-contraction interval. These changes led to impaired voiding function by induction of IC ($P < 0.05$). However, PDRN treatment decreased the IC-induced contraction pressure and contraction time with increased inter-contraction interval ($P < 0.05$). To assess the relevance of PDRN efficacy to the adenosine A_{2A} receptor, combination treatment of DMPX with PDRN eliminated the improving effect of PDRN on voiding function such as contraction pressure, contraction time, and inter-contraction interval.

Bladder Weight and Inflammatory Score

Bladder weight and histological changes of cyclophosphamide-induced IC are shown in Figure 3A–C. Induction of IC by cyclophosphamide injection caused epithelial detachment and erosion and infiltration of inflammatory cells (marked with black arrows). In addition, lamina propria showed edematous stroma and irregular ectasia vessels (marked with blue arrows).

These results increased bladder weight and inflammatory score ($P < 0.05$). However, PDRN treatment was observed to decrease inflammatory score and bladder weight due to the appearance of normal mucosal structure, improved inflammatory cell infiltration, and reduced swelling of the bladder tissue ($P < 0.05$). To assess the relevance of PDRN efficacy to the adenosine A_{2A} receptor, combination treatment of DMPX with PDRN eliminated the reducing effect of PDRN on bladder weight and inflammation score.

Pro-Inflammatory Cytokines

The expression levels of TNF-α and IL-1β are shown in Figure 4. Induction of IC by cyclophosphamide injection increased the expression levels of TNF-α and IL-1β in the bladder tissue ($P < 0.05$). In contrast, treatment with PDRN reduced the expression levels of TNF-α and IL-1β in the bladder tissue ($P < 0.05$). To assess the relevance of PDRN efficacy to the adenosine A_{2A} receptor, combination treatment of DMPX with PDRN eliminated the inhibitory effect of PDRN on TNF-α and IL-1β production.

NF-κB/MAPK Signaling Pathway

Activation of NF-κB and phosphorylation of MAPK signaling pathways are shown in Figure 5. Induction of IC by cyclophosphamide injection enhanced NF-κB expression in the bladder tissue by stimulating the phosphorylation of IκB-α ($P < 0.05$). In contrast, PDRN treatment decreased IκB-α phosphorylation and suppressed NF-κB expression in the bladder tissue ($P < 0.05$), suggesting that PDRN treatment inactivated NF-κB. Induction of IC by cyclophosphamide injection enhanced phosphorylation of MAPK signaling pathway such as ERK, JNK, and p-38. PDRN treatment reduced phosphorylation of the MAPK signaling pathway in the bladder tissue ($P < 0.05$). To assess the relevance of PDRN efficacy to the adenosine A_{2A} receptor, combination treatment of DMPX with PDRN eliminated the inhibitory effect of PDRN on phosphorylation of IκB-α, ERK, JNK and p38 (Supplement 1).

cAMP, p-CREB, and p-PKA Expressions

The cAMP level measured by ELISA and the ratio of p-CREB/CREB and p-PKA/PKA detected by Western blotting are shown in Figure 6A–C. Induction of IC by cyclophosphamide injection resulted in a decrease in cAMP level in the bladder ($P < 0.05$), whereas PDRN treatment resulted in increased cAMP level in the bladder tissue ($P < 0.05$). Induction of IC by cyclophosphamide

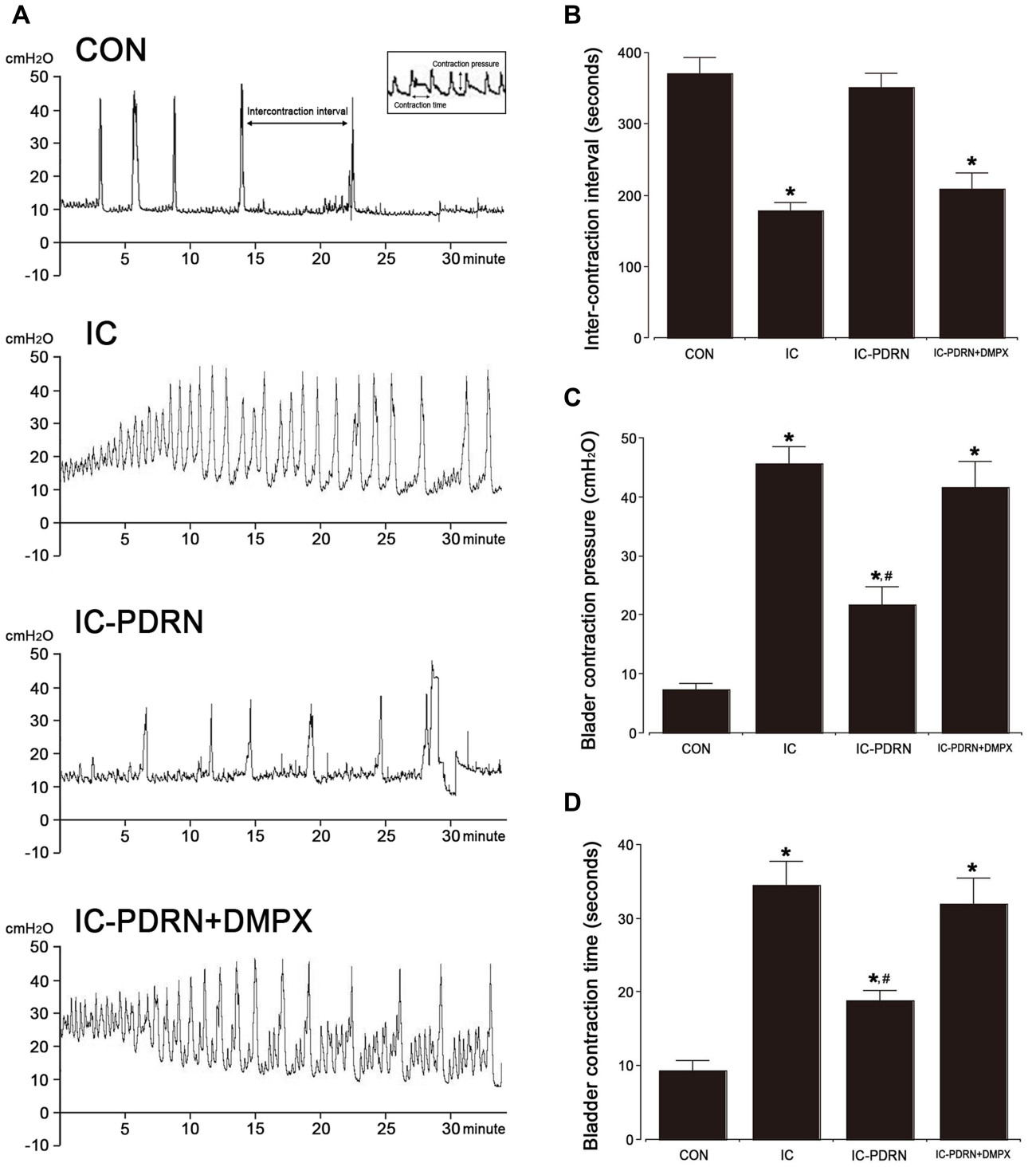

Figure 2 Voiding functions. (**A**) The inter-contraction pressure, contraction pressure and time change as recorded by cystometry. (**B**) Comparison of inter-contraction interval in each group. (**C**) Comparison of contraction pressure in each group, (**D**) Comparison of contraction time in each group. *Indicates $P < 0.05$ when compared with the control group. #Indicates $P < 0.05$ compared with the IC-induced group.

injection increased the phosphorylation of CREB and PKA ($P < 0.05$), in contrast, PDRN treatment suppressed the phosphorylation of CREB and PKA in the bladder tissue ($P < 0.05$). To assess the relevance of PDRN efficacy to the adenosine A_{2A} receptor, combination treatment of DMPX with PDRN eliminated increasing effect of PDRN on cAMP level and abolished suppressing effect of PDRN on CREB and PKA phosphorylation.

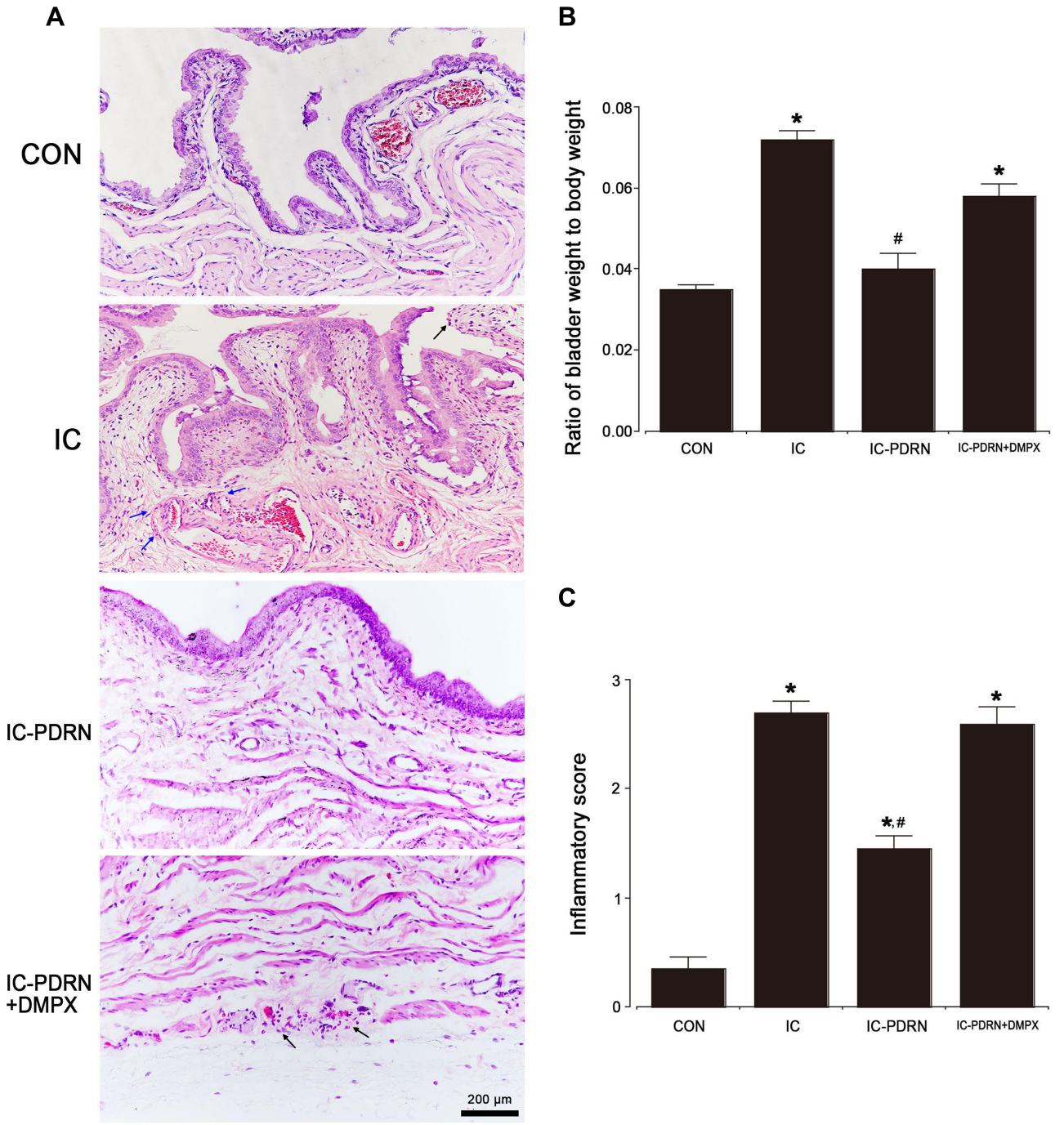

Figure 3 Ratio of bladder weight to body weight and inflammatory score. (**A**) Histological changes in the bladder tissue. (**B**) Ratio of bladder weight to body weight in each group. (**C**) Inflammatory score in each group. Black arrows indicate epithelial detachment, erosion, and inflammatory cells. Blue arrows indicate edematous stroma and irregularly ectasia vessels. *Indicates $P < 0.05$ when compared with the control group. #Indicates $P < 0.05$ compared with the IC-induced group.

DNA Fragmentation and Bax/Bcl-2 Ratio

DNA fragmentation was detected by TUNEL staining and expressions of Bax and Bcl-2 were measured by Western blotting (Figure 7A and B). Induction of IC by cyclophosphamide injection increased DNA fragmentation and Bax/Bcl-2 ratio in the bladder ($P < 0.05$). However, PDRN treatment inhibited DNA fragmentation and Bax/Bcl-2 ratio in the bladder ($P < 0.05$). To assess the relevance of PDRN efficacy to the adenosine A_{2A} receptor, combination treatment of DMPX with PDRN eliminated decreasing effect of PDRN on DNA fragmentation and Bax/Bcl-2 ratio.

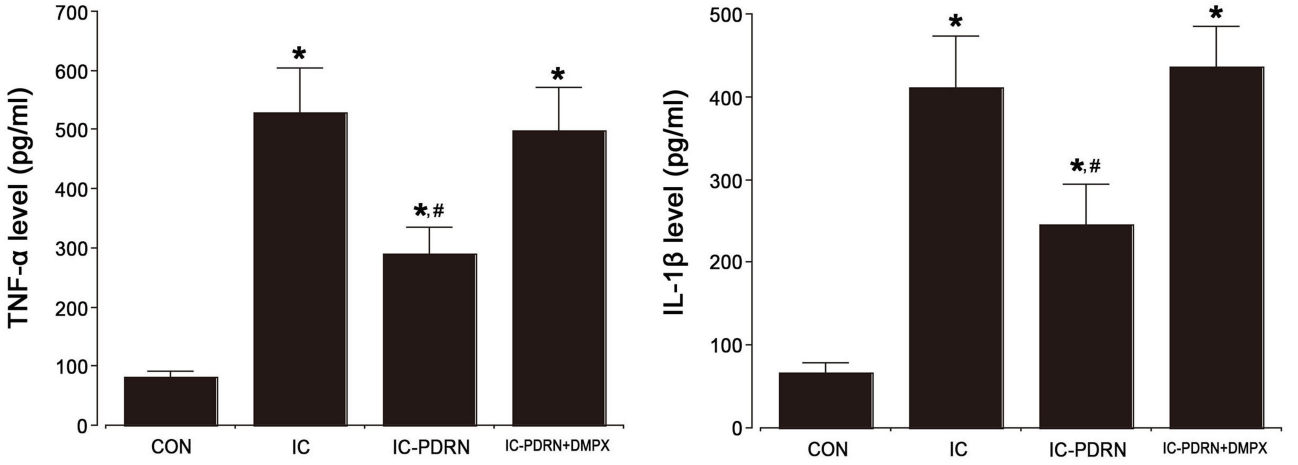

Figure 4 Pro-inflammatory cytokines. Left: Tumor necrosis factor (TNF)-α level in the bladder tissue. Right: Interleukin (IL)-1β level in the bladder tissue. *Indicates P < 0.05 when compared with the control group. #Indicates P < 0.05 compared with the IC-induced group.

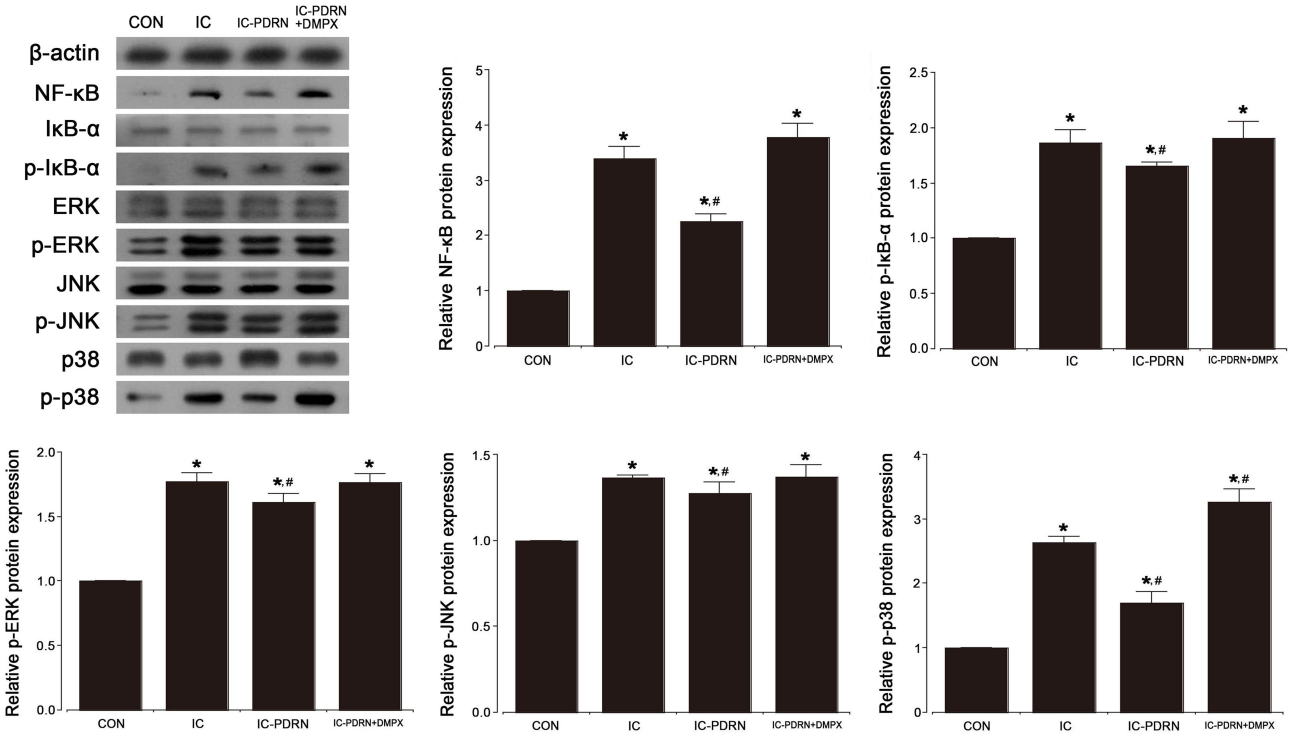

Figure 5 Nnuclear factor (NF)-κB, NF-κB inhibitor-α (IκB-α), and cascade factors of mitogen-activated protein kinase (MAPK). Upper left panel: Representative expressions of NF-κB, NF-κB inhibitor (IκB)-α, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p-38. Upper right panel: Relative expression of NF-κB and relative ratio of phosphorylated IκB-α (p-IκB-α) to IκB-α. Lower panel: Relative ratio of phosphorylated ERK (p-ERK) to ERK, phosphorylated JNK (p-JNK) to JNK, and phosphorylated p-38 (p-p-38) to p-38. *Indicates P < 0.05 when compared with the control group. #Indicates P < 0.05 compared with the IC-induced group.

Discussion

The symptoms of the IC patients are very diverse, and most of them show voiding problems such as urinary frequency and urgency as well as bladder associated pain or discomfort. Cyclophosphamide-induced IC model is widely used to assess the inflammatory change of the bladder and related micturition dysfunction.[29] Cyclophosphamide injection also causes urothelium hyperplasia and induces inflammatory response.[2] In the current study, the cystometry to measure the functional change of the bladder showed increased contraction pressure and contraction time, whereas decreased

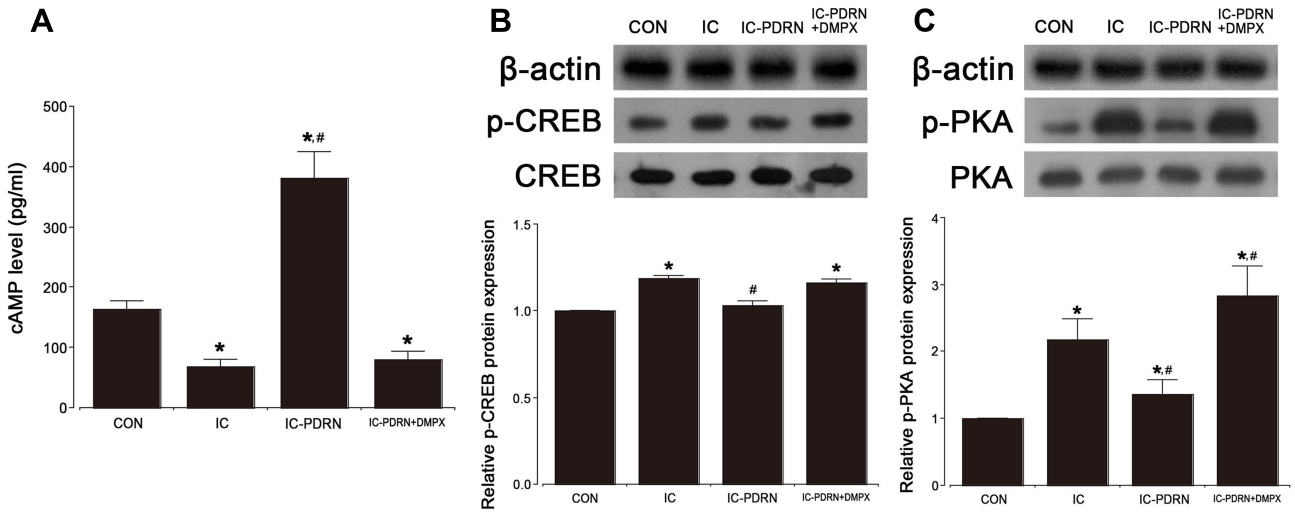

Figure 6 Cyclic adenosine-3′,5′-monophosphate (cAMP), cAMP response element-binding protein (CREB) and protein kinase A (PKA). (**A**) cAMP level in the bladder tissue. (**B**) Relative ratio of phosphorylated CREP (p-CREB) to CREB in bladder tissue. (**C**) Relative ratio of phosphorylated PKA (p-PKA) to PKA in bladder tissue. *Indicates $P < 0.05$ when compared with the control group. #Indicates $P < 0.05$ compared with the IC-induced group.

intercontraction interval. Cyclophosphamide injections caused mucosal edema and inflammatory cell infiltration, which increased bladder weight and inflammation score. Current findings were similar to previous studies showing bladder tissue damage and impaired urination due to cyclophosphamide.[2,23,29]

Inflammation serves to aggravate the symptoms of IC, especially, the production of pro-inflammatory cytokines by IC caused voiding dysfunction and bladder pain or discomfort.[3,5,7] In the current study, the deleterious effect of cyclophosphamide on the bladder may be attributed to increased secretion of TNF-α and IL-1β in the bladder. Our experiment results showed that overproduction of pro-inflammatory cytokines aggravated the progress of IC. Clinically, because pro-inflammatory biomarkers were significantly increased in IC patients,[5,30] suppression on pro-inflammatory cytokines is an important treatment strategy for IC.

Since the secretion of pro-inflammatory cytokines is inhibited by stimulation of adenosine A_{2A} receptor in monocytes and macrophages,[31,32] the adenosine A_{2A} receptor agonist PDRN has been proposed as a treatment for inflammatory diseases.[18,20,22] In the current study, PDRN treatment reduced TNF-α and IL-1β expressions in IC-induced rats, suggesting that decrease of pro-inflammatory cytokines by PDRN plays a critical step in IC treatment strategy.

Inflammation is an immune response to damage, and the MAPK signaling pathway primarily regulates the inflammation process.[33,34] Phosphorylation of MAPK signaling pathway is increased in cyclophosphamide-induced IC.[7,33,35] It is also well known that MAPK controls NF-κB.[7] The NF-κB transcription factor plays a crucial role in the inflammatory process and is activated by the breakdown of the IκB-α protein.[36] The activated NF-κB complex translocates to the nucleus to produce pro-inflammatory cytokines, increase of which is involved in the progression of inflammatory diseases such as IC.[37] In the current study, administration of cyclophosphamide enhanced MAPK cascade phosphorylation in the bladder tissue, and this response eventually induces IκB-α activation, increasing the activity of NF-κB. However, PDRN treatment reduced the activation of IκB-α and NF-κB in the bladder tissue by inhibiting phosphorylation of MAPK cascade.

Stimulation-induced activation of the adenosine A_{2A} receptor increases the concentration of cAMP in cells, thereby inhibiting inflammatory neutrophils.[38] The cAMP-PKA signaling pathway is activated by the adenosine A_{2A} receptor to accelerate phosphorylation of CREB and inhibits pro-inflammatory cytokines through regulation of NF-κB.[39] In addition, increased cAMP level suppresses the phosphorylated form of the MAPK cascade in stimulated cells.[38,40] In the current study, PDRN treatment increased cAMP level and adenosine A2A receptor in the bladder tissue of cyclophosphamide-induced IC rats (Supplement 2). Enhancement of cAMP level in the bladder by PDRN treatment suppressed MAPK phosphorylation and NF-κB activation.

Increased cAMP in urinary tract cells is known to induce the release of anti-apoptotic proteins to inhibit

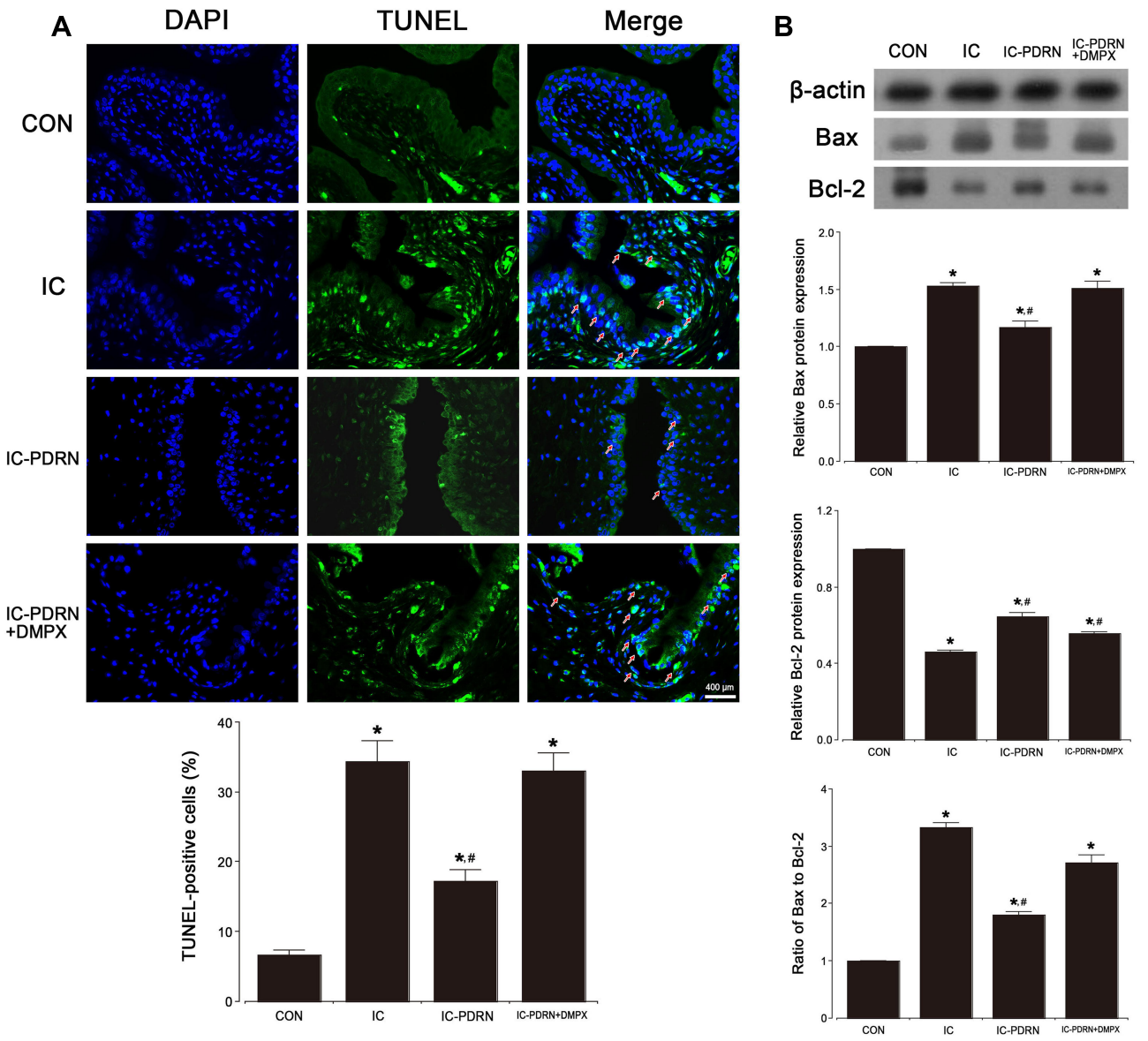

Figure 7 Apoptosis in the bladder tissue. (**A**) Percentages of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)-positive (red arrows) cells in the bladder tissue. (**B**) Relative ratio of Bax to Bcl-2 in bladder tissue. *Indicates $P < 0.05$ compared with the control group. #Indicates $P < 0.05$ compared with the IC-induced group.

apoptosis.[41] Increased cAMP concentration plays an important role in suppressing apoptotic cell death. Since apoptosis is involved as a critical process in the pathogenesis of IC,[4] suppressing apoptosis is the key to IC treatment. In the current study, cyclophosphamide-induced IC rats treated with PDRN showed low expression of DNA fragmentation and a low Bax to Bcl-2 ratio.

Conclusion

In this study, PDRN treatment improved voiding dysfunction by suppressing inflammation and apoptosis in IC rats through increased cAMP concentration in the bladder tissue. On the other hand, co-treatment of adenosine A_{2A} receptor antagonist DMPX with adenosine A_{2A} receptor agonist PDRN did not reduce inflammation and apoptosis, resulting in no improvement in voiding dysfunction. With the current result, PDRN may be expected to be effective treatment for bladder inflammation. As a limitation of the study, pain is an important feature observed in IC patients, but was not measured in this study. For this reason, we believe that it is insufficient to demonstrate the efficacy of PDRN for pain in the IC model. Therefore, further research is needed to evaluate pain improvement.

Data Sharing Statement

All data generated in this study are included in this manuscript.

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Authorship Contributions

Ko I.G: Conceptualization, Project administration, Writing-original draft, Jin J.J: Methodology, Formal analysis, Hwang L: Methodology, Formal analysis, Kim S.H: Methodology, Investigation, Resources, Kim C.J: Supervision, Project administration, Won K.Y: Methodology, Formal analysis, Na Y.G: Supervision, Resources, Kim K.H: Investigation, Writing- review and editing, Kin S.J: Supervision, Project administration, Writing- review and editing. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors have no conflicts of interest for this work to declare.

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