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# Expression and Regulation of Brain Natriuretic Peptide and Natriuretic Peptide Receptor A (NPR-A) in L6–S1 Dorsal Root Ganglia in a Rat Model of Chronic Nonbacterial Prostatitis

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Statistical Analysis C  
Data Interpretation D  
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**Background:** The study aimed to investigate the expression of brain natriuretic peptide (BNP) and natriuretic peptide receptor A (NPR-A) in L6–S1 dorsal root ganglia (DRG) in a rat model of chronic nonbacterial prostatitis (CNP).

**Material/Methods:** One hundred specific pathogen-free (SPF) male Sprague–Dawley rats were randomly divided into a control group (N=50) and a study group (N=50). The control group underwent prostatic injection of 0.1 ml of normal saline on days 3, 7, 10, 14, and 28. The study group, or rat model of CNP, underwent prostatic injection of 0.1 ml of complete Freund's adjuvant on days 3, 7, 10, 14, and 28. At the end of the study, the rats were euthanized, and the prostate tissues and L6–S1 DRG were removed. Histology was performed on the prostate tissue from the rats in the study group and control group. Real-time fluorescence-based quantitative polymerase chain reaction (PCR) and Western blot were used to study the expression of BNP and NPR-A mRNA and protein in the DRG from the rats in the study group and control group.

**Results:** In the rat model of CNP, the expression of BNP and NPR-A were significantly increased in L6–S1 DRG compared with the controls.

**Conclusions:** In a rat model of CNP, the increased expression of BNP and NPR-A in L6–S1 DRG may have a role in pain signaling pathways associated with chronic prostatitis.

**MeSH Keywords:** **Chronic Pain • Natriuretic Peptide, Brain • Prostatitis**

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## Background

Chronic nonbacterial prostatitis (CNP), also known as chronic pelvic pain syndrome, is a common condition in men that includes long-term symptoms of chronic pain or discomfort in the pelvic region due to inflammation and fibrosis of the prostate, lower urinary tract, and pelvis [1–3]. Approaches to the treatment of CNP have focused on the prevention or control of inflammation and fibrosis [4]. However, although the pathogenesis of CNP remains unclear, the condition is associated with chronic neuropathic pain, pain due to inflammation and immune factors [5]. The pain associated with CNP may be associated with abnormal functions of complex conduction pathways and neuromodulation mechanisms [6]. It has also been proposed that the symptoms of CNP are similar to the symptoms associated with secondary lesions involving the spinal cord at level L6–S1 that is associated with the prostate [7,8].

Brain natriuretic peptide (BNP) is a member of the natriuretic peptide family (NPF) that activates the expression of natriuretic peptide receptor-A (NPR-A) [9]. Recent studies have shown that BNP and NPR-A are also involved in the regulation of pain associated with inflammation [10].

Therefore, this study aimed to investigate the expression of brain natriuretic peptide (BNP) and natriuretic peptide receptor A (NPR-A) in the L6–S1 dorsal root ganglia (DRG) in a rat model of chronic nonbacterial prostatitis (CNP) to determine the possible pathways involved.

## Material and Methods

### Experimental animals

The local Ethics Committee approved this study, and approval for the use of animals in this study was obtained (Animal License No. SCXK [Hunan] 2013–0004). One hundred specific pathogen-free (SPF) male Sprague-Dawley rats with an average body weight of  $220 \pm 30$  g were used. The rats were randomly divided into a control group (N=50) and a study group (N=50). The control group underwent injection of 0.1 ml of normal saline into the lateral lobe of the prostate on days 3, 7, 10, 14, and 28. The study group, or rat model of chronic nonbacterial prostatitis (CNP), underwent prostatic injection of 0.1 ml of complete Freund's adjuvant into the lateral lobe of the prostate on days 3, 7, 10, 14, and 28. Both groups of rats were routinely fed and observed for one week before the study began. The rats were free to drink water and eat food during the whole experimental period. The rats were housed in an Intelligent artificial climate box, RXZ-380C (Jiangnan. Instrument Factory, Ningbo, China) with the temperature maintained at  $20\text{--}25^\circ\text{C}$ , and relative humidity of  $55 \pm 5\%$ .

### Equipment and reagents

The equipment used in this study included an Olympus BX51 light microscope (Olympus Corporation, Tokyo, Japan); complete Freund's adjuvant (Sigma-Aldrich, St. Louis MO, USA); reverse transcription kit (K1699) (Thermo Fisher Scientific, Waltham, MA, USA); a fluorescence-based quantitative polymerase chain reaction (PCR) kit (RR420A) (TaKara, Tokyo, Japan); PCR primers (GenScript, Nanjing, China); anti-BNP antibody (ab19645) (Abcam, Cambridge, UK); anti-NPR-A antibody (GTX109810) (Genetex, Irvine, CA, USA); anti-GAPDH antibody (ab9485) (Abcam, Cambridge, UK); secondary antibody (ab6789) (Abcam, Cambridge, UK); ECL kit (34094) (Thermo Fisher Scientific, Waltham, MA, USA). Other reagents used were obtained locally and were of pure analytical grade.

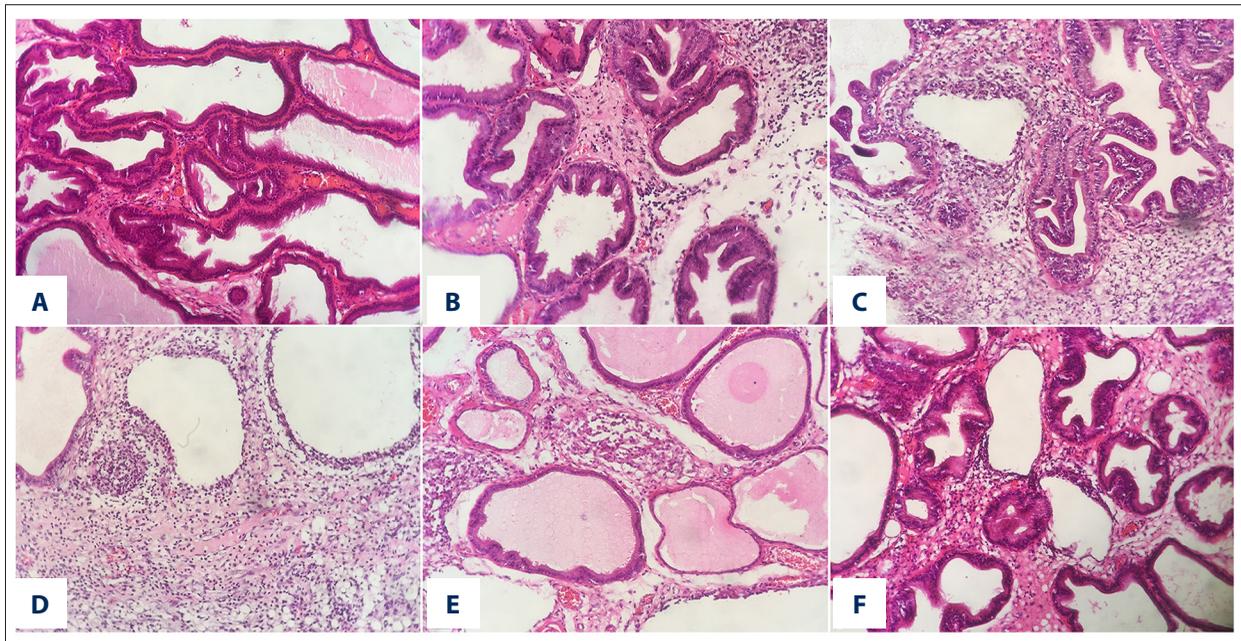
### The rat model of CNP

The rat model of CNP was developed by injection of the rat prostate gland with 0.1 mL of complete Freund's adjuvant. Under aseptic conditions, a medial longitudinal incision was made in the perineum to expose the prostate gland. The experimental or study group (N=50) underwent injection with 0.1 mL of complete Freund's adjuvant into the left lobe of the prostate gland on days 3, 7, 10, 14, and 28. The control group (N=50) was injected with 0.1 mL of normal saline solution on days 3, 7, 10, 14, and 28. The skin incisions were sutured in layers with absorbable sutures. The rats were then returned to the Intelligent artificial climate box (RXZ-380C) (Jiangnan. Instrument Factory, Ningbo, China).

### Sampling of rat prostate tissues and L6–S1 dorsal root ganglia (DRG)

At the end of the study, the rats were euthanized, and the prostate tissues and L6–S1 DRG were removed for light microscopy. Rats in the study group and control group were injected intraperitoneally with 10% chloral hydrate (0.4 ml/100 g body weight) and sacrificed by cervical dislocation. The prostate glands were removed and fixed in 4% formaldehyde solution, embedded in paraffin wax, and sectioned onto glass slides for further study.

The rat L6–S1 DRG were anatomically identified and removed by first removing a vertebral disc, spinous process, and transverse process to expose the L6–S1 nerves on both sides of the spine. The enlarged L6–S1 DRG were removed and placed in a sterilized cryopreservation tube. The tube was quickly labeled, placed in the liquid nitrogen and transferred to a  $-80^\circ\text{C}$  refrigerator for storage.



**Figure 1.** Photomicrographs of the histology of the prostate tissue in the Sprague-Dawley rat model of chronic nonbacterial prostatitis (CNP) (study group) and the control (sham) group. **(A)** Histology of the prostate in the control (sham) rat group shows normal and regularly arranged prostate glands with minimal edema or inflammation edema, and no inflammatory cell infiltrates in the interstitial tissue. Hematoxylin and eosin (H&E). Magnification  $\times 200$ . **(B)** Histology of the prostate in the control (sham) rat group at day 3 following injection of normal saline shows mild inflammation, vascular dilatation, and congestion, with mild infiltration of neutrophils and lymphocytes. H&E. Magnification  $\times 200$ . **(C)** Histology of the prostate in the study group (the rat model of CNP) at day 7 shows moderate inflammation with loss of glands with neutrophils and lymphocytes in the perivascular and interstitial tissues. H&E. Magnification  $\times 200$ . **(D)** Histology of the prostate in the study group (the rat model of CNP) at day 10 shows moderate and severe inflammation with loss of glands with neutrophils and lymphocytes in the perivascular and interstitial tissues. H&E. Magnification  $\times 200$ . **(E)** Histology of the prostate in the control (sham) rat group at day 14 following injection of normal saline shows mild inflammation, vascular dilatation, and congestion, with mild infiltration of neutrophils and lymphocytes. H&E. Magnification  $\times 200$ . **(F)** Histology of the prostate in the control (sham) rat group at day 28 following injection of normal saline shows mild inflammation, vascular dilatation, and congestion, with mild infiltration of neutrophils and lymphocytes. H&E. Magnification  $\times 200$ .

### Histology of the rat prostate and detection of BNP and NPR-A in L6–S1 DRG

Serial tissue sections of rat prostate were cut at a thickness of 4  $\mu\text{m}$  from the prostate tissue wax block onto glass slides. Tissue sections were stained with hematoxylin and eosin (H&E). The morphological changes of the prostate glands and the degree of inflammation were assessed by light microscopy at a magnification of  $\times 200$ . The expression of BNP and NPR-A in L6–S1 DRG tissue was determined by real-time fluorescence-based quantitative polymerase chain reaction (PCR) and Western blot.

### Statistical analysis

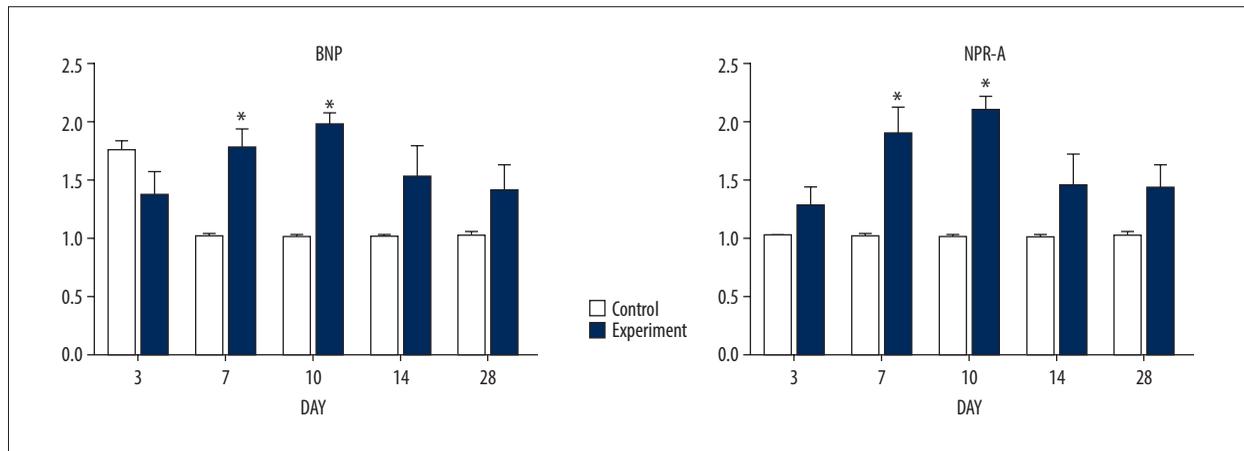
The PCR and Western blot data were analyzed by SPSS version 17.0 software (IBM, Chicago, IL, USA). Comparative analysis of the data obtained from each group was performed using one-way analysis of variance (ANOVA) and the least significant

difference (LSD) test. Experimental data were expressed as the mean  $\pm$  standard deviation (SD). A P-value  $< 0.05$  was considered to be statistically significant.

## Results

### Histology of the rat prostate tissue in the control (sham) rat group and the rat model chronic nonbacterial prostatitis (CNP)

Figure 1 shows the histology of the normal rat prostate tissue from the control (sham) rat group, with regular prostate glands, no edema, or inflammation. Prostate tissue in the rat model of CNP injected with complete Freund's adjuvant at day 3 showed that there was mild inflammation with edema and infiltration of neutrophils and lymphocytes, which increased in severity at day 7. Vascular changes included vascular leak and perivascular and interstitial infiltration of neutrophils and



**Figure 2.** Real-time fluorescence-based quantitative polymerase chain reaction (PCR) for mRNA expression of brain natriuretic peptide (BNP) and natriuretic peptide receptor A (NPR-A) in L6–S1 dorsal root ganglia (DRG) in a rat model of chronic nonbacterial prostatitis (CNP). Real-time PCR analysis shows that the mRNA expression of BNP and NPR-A increased in L6–S1 DRG of rats in the experimental group, compared with the control group. The mRNA expression of BNP and NPR-A in L6–S1 DRG gradually increased and peaked at day 7 and day 10 in the study group (n=10) (\*  $p < 0.05$ ).

lymphocytes by day 10 in the CNP study group. In the control group, the histological appearance of the prostate was similar at day 3, day 7, day 14, and day 28.

#### Expression of brain natriuretic peptide (BNP) and natriuretic peptide receptor A (NPR-A) mRNAs in rat L6–S1 dorsal root ganglia (DRG)

The mRNAs expression level of BNP and NPR-A in L6–S1 DRG in the control group and the experimental group at day 3, day 7, day 10, day 14, and day 28 were measured by real-time fluorescence-based quantitative polymerase chain reaction (PCR). The expression level of BNP and NPR-A mRNA was analyzed by using GAPDH as the internal reference gene. The expression levels of BNP and NPR-A mRNA in the DRG of the rats in the experimental group were significantly higher than the DRG in the rats in the control group at day 3, day 7, day 10, day 14, and day 28 ( $P < 0.05$ ) (Figure 2).

#### Expression of BNP and NPR-A protein in rat L6–S1 DRG

The protein expression level of BNP and NPR-A in the L6–S1 DRG of the control group and the experimental group was measured by Western blot at day 3, day 7, day 10, day 14, and day 28. The expression level of BNP and NPR-A in the study group was significantly higher than in the control group day 3, day 7, day 10, day 14, and day 28 ( $P < 0.05$ ) (Figure 3).

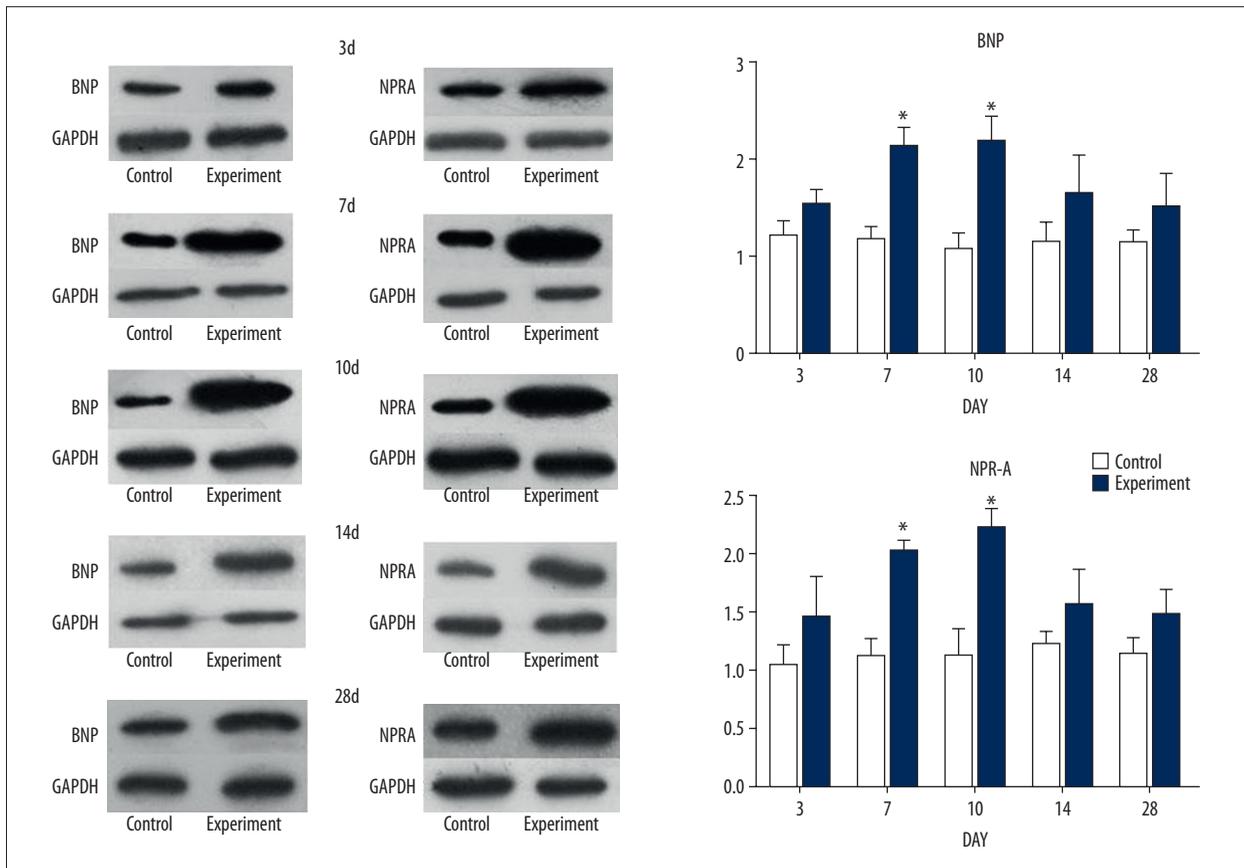
## Discussion

Chronic nonbacterial prostatitis (CNP) is a common urological condition in men that is also known as chronic pelvic pain

syndrome. Pain is the most common symptom and can be chronic and severe, leading to impaired quality of life for patients [11]. The findings from recent studies support that the pain associated with CNP has multiple causes that lead to neuropathic pain from the activation of astrocytes and changes in dorsal root ganglion cells (DRGs) [12].

Brain natriuretic peptide (BNP) is a polypeptide that binds to natriuretic peptide receptor A (NPR-A) and increases the expression of intracellular cyclic guanosine monophosphate (cGMP) [13]. Protein kinase G (PKG) is also a cellular protein that has an important role in cGMP-mediated signal transduction pathways and regulation of intracellular  $Ca^{2+}$ . Large-conductance, calcium-activated potassium (BKCa) channels are potassium channels that are gated by  $Ca^{2+}$ , which can alter the permeability of the cell membrane to potassium that affects the excitability of nerve cells, the release of neurotransmitters, and repolarization of action potentials [13]. BKCa channels participate in repolarization and hyperpolarization of action potentials at the synaptic terminal end of the neuronal cell bodies to regulate neuron excitability [13]. Nerve injury inhibits the expression of BKCa in the dorsal root of the spinal cord, resulting in the release of cellular factors associated with the sensation of neurogenic pain [14].

The nociceptive neurons in the DRG are involved in the first stage of the pain conduction pathway. Nociceptive stimuli release excitatory neurotransmitters from the neuronal afferent fibers, resulting in the transmission of the sensation of pain to the spinal cord and brain. Zhang et al. showed that inflammation of peripheral tissues could significantly increase the expression of the BNP and NPR-A genes in the DRG of nociceptive neurons [10]. The upregulation of BNP and NPR-A can



**Figure 3.** Western blot for protein expression of brain natriuretic peptide (BNP) and natriuretic peptide receptor A (NPR-A) in L6–S1 dorsal root ganglia (DRG) in a rat model of chronic nonbacterial prostatitis (CNP). Western blot analysis shows that the protein expression levels of BNP and NPR-A increased in L6–S1 DRG of rats in the experimental group, compared with the control group. Protein expression of BNP and NPR-A in L6–S1 DRG gradually increased and peaked in the day 7 group and day 10 group (n=10). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. (\*  $p < 0.05$ ).

increase the gating frequency of BKCa, reducing the excitability of nociceptive neurons and resulting in pain suppression [15]. Therefore, BNP secreted by the nociceptive sensory afferent fibers acts as an inhibitory agent in the regulation of excitatory synaptic transmission by activating presynaptic NPR-A [15]. BNP and NPR-A are also involved in inflammatory pain regulation mechanisms, which means that activation of the BNP-NPR-A pathway in nociceptive neurons has potential as a novel analgesic strategy [15]. The activation of the BNP and NPR-A signal transduction pathways in the nociceptive neurons might be an effective approach to pain management. Previous studies in rat models of inflammation and pain have identified possible pathways for neuronal excitability and activation of nociceptors that may be targeted to inhibit pain associated with inflammation [16].

Zhang et al. previously showed that the BNP/NPR-A signal transduction pathways had a role in the inhibition of the pain signal transmission (nociception) [10]. In the present study, the rat model of CNP was developed using an injection of complete

Freund's adjuvant into the lobe of the prostate gland at on days 3, 7, 10, 14, and 28. Histology of the prostate tissue in the rat model showed that inflammatory changes reached a peak at day 7. Real-time fluorescence-based quantitative polymerase chain reaction (PCR) and Western blot showed that expression of BNP and NPR-A mRNA and protein in the L6–S1 dorsal root ganglia (DRG) in the rat model of CNP were significantly higher than that of control group and correlated with the degree of inflammation found in the rat prostate gland. These results suggest that BNP and NPR-A had a close relationship with chronic prostatitis in this rat model of CNP and were consistent with findings previously reported by Zhang et al. [10].

Prolonged stimulation of sensory neurons can increase neuronal excitability after pain sensitization that may reduce the response to harmful stimuli [16,17]. In this study, in the rat model of CNP, there was no significant difference in the expression of BNP and NPR-A between day 14 and day 28 and the control group. This finding suggested that during the day 14 and day 28 of inflammation of the rat prostate gland, there might

have been sensitization to pain, due reduced inhibitory factors reflected by the expression levels of BNP and NPR-A in the 14d and 28d groups, which were not significantly different from the control rat group.

## Conclusions

The study aimed to investigate the expression of brain natriuretic peptide (BNP) and natriuretic peptide receptor A (NPR-A)

in L6–S1 dorsal root ganglia (DRG) in a rat model of chronic nonbacterial prostatitis (CNP). The findings showed that BNP and NPR-A were upregulated in L6–S1 DRG in the rat model of CNP, which suggests that BNP/NPR-A signal transduction pathways may be involved in the regulation of pain in chronic prostatitis.

## Conflict of interest

None.

## References:

1. Delavierre D, Rigaud J, Sibert L et al: Symptomatic approach to chronic prostatitis/chronic pelvic pain syndrome. *Prog Urol*, 2010; 20: 940–53
2. Korkmaz S, Karadag MA, Hamamcioglu K et al: Electrophysiological identification of central sensitization in patients with chronic prostatitis. *Urol J*, 2015; 12: 2280–84
3. Shan P, Lu Z, Ye L et al: Effect of tripterygium wilfordii polyglycoside on experimental prostatitis caused by ureaplasma urealyticum in rats. *Med Sci Monit*, 2016; 22: 3722–26
4. Kulchavenya EV, Shvetsova OP, Breusov AA: Rationale of use and effectiveness of Longidaza in patients with chronic prostatitis. *Urologiia*, 2018; 4: 64–71
5. Pontari MA, Ruggieri MR: Mechanisms in prostatitis/chronic pelvic pain syndrome. *J Urol*, 2008; 179 (5 Suppl.): S61–67
6. Park JS, Jin MH, Hong CH et al: Neurologic mechanisms underlying voiding dysfunction due to prostatitis in a rat model of nonbacterial prostatic inflammation. *Int Neurourol J*, 2018; 22(2): 90–98
7. Ishigooka M, Nakada T, Hashimoto T et al: Spinal substance P immunoreactivity is enhanced by acute chemical stimulation of the rat prostate. *Urology*, 2002; 59: 139–44
8. Chen Y, Wu X, Liu J et al: Distribution of convergent afferents innervating bladder and prostate at dorsal root Ganglia in rats. *Urology*, 2010; 76: 764. e1–6
9. Huntley BK, Sandberg SM, Noser JA et al: BNP-induced activation of cGMP in human cardiac fibroblasts: interactions with fibronectin and natriuretic peptide receptors. *J Cell Physiol*, 2006; 209: 943–49
10. Zhang FX, Liu XJ, Gong LQ et al: Inhibition of inflammatory pain by activating B-type natriuretic peptide signal pathway in nociceptive sensory neurons. *J Neurosci*, 2010; 30: 10927–38
11. Zhao FL, Yue M, Yang H et al: Health-related quality of life in Chinese patients with chronic prostatitis/chronic pelvic pain syndrome. *Qual Life Res*, 2010; 19: 1273–83
12. Schaeffer AJ: Etiology and management of chronic pelvic pain syndrome in men. *Urology*, 2004; 63(3 Suppl. 1): 75–84
13. Deenadayalu V, Puttabyatappa Y, Liu AT et al: Testosterone-induced relaxation of coronary arteries: activation of BKCa channels via the cGMP-dependent protein kinase. *Am J Physiol Heart Circ Physiol*, 2012; 302(1): H115–23
14. Chen SR, Cai YQ, Pan HL: Plasticity and emerging role of BKCa channels in nociceptive control in neuropathic pain. *J Neurochem*, 2009; 110: 352–62
15. Kallenborn-Gerhardt W, Schmidtke A: A novel signaling pathway that modulates inflammatory pain. *J Neurosci*, 2011; 31: 798–800
16. Dubner R, Ruda MA: Activity-dependent neuronal plasticity following tissue injury and inflammation. *Trends Neurosci*, 1992; 15: 96–103
17. Woolf CJ, Ma Q: Nociceptors – noxious stimulus detectors. *Neuron*, 2007; 55: 353–64