in vivo *38*: 691-698 (2024)
doi:10.21873/invivo.13490

Involvement of NOTCH1-mediated Microglia Activation in Neuromodulation of Chronic Prostatitis-related Pain

HENG ZHANG[1], RUIFEN GU[2], JISHENG LUO[1], CHUANHUA ZHONG[1] and JINHONG PAN[1]

[1]*Department of Urology, Guiqian International General Hospital, Guiyang, P. R. China;*
[2]*Department of Pathology, Guiqian International General Hospital, Guiyang, P. R. China*

Abstract. *Background/Aim: This study aimed to investigate the role of NOTCH receptor 1 (NOTCH1)-mediated activation of microglia in the L5-S2 spinal dorsal horn in chronic prostatitis pain. Materials and Methods: Rats were divided into chronic prostatitis (CP) group and control group. Complete Freund's adjuvant was injected into the prostate, and prostate pathology and pain-related behavior were monitored to assess the successful establishment of the CP-related pain model. The dorsal horn of the L5-S2 spinal cord was collected for the detection of ionized calcium-binding adapter molecule 1 (IBA-1) and NOTCH1 expression by quantitative real time polymerase chain reaction and the detection of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) by enzyme-linked immunosorbent assay. Electrical excitability was assessed with whole-cell patch clamp. In addition, NOTCH1 receptor inhibitor or inhibitor of microglial cell activation was injected into the subarachnoid space, and the pro-inflammatory cytokines in the spinal cord were detected. Results: In the CP group, the expression of NOTCH1, IBA-1, TNF-α and IL-1β began to increase at 4 days, peaked at 12 days, and began to decline at 24 days, and it was significantly higher than in the control group (p<0.01). Inhibition of microglia or NOTCH1 receptor markedly reduced the content of TNF-α and IL-1β in the spinal cord (p<0.05). At 4, 12 and 24 days, the amplitude and frequency of neuronal action potential increased and the threshold decreased markedly as compared to the control group (p<0.05), and spontaneous action potential was noted. Conclusion: NOTCH1 mediates the activation of microglia in the L5-S2 spinal cord, leading to the secretion of inflammatory factors and enhanced electrical excitability of neurons, which is related to persistent and refractory chronic prostatitis-related pain.*

Correspondence to: Chuanhua Zhong and Jinhong Pan, Department of Urology, Guiqian International General Hospital, Guiyang, 550018, P.R. China. E-mail: zch@guiqianhospital.com (CZ); pjh@guiqianhospital.com (JP)

Key Words: Prostatitis-related pain, spinal cord, NOTCH1, microglia, neuron.

Chronic prostatitis (CP) is a persistent and refractory disease seen at the Department of Urology, and often affects male reproduction. Currently, the incidence of CP is about 8.2% in males and patients with CP account for about 30% of outpatients. Moreover, type IIIA chronic aseptic prostatitis/chronic pelvic pain syndrome (CPPS) (type IIIb) accounts for about 90% of CP in clinical practice (1). However, the etiology of CP is still poorly understood, and current treatments for CP have limited efficacy. Long-lasting disease in these patients may cause severe somatopsychic disturbance which is still refractory to available treatments (2). Therefore, the diagnosis and treatment of CP have been a great challenge in the field of urology and the pathogenesis of CP has been a hot topic in this field. CP-related pain often manifests as persistent visceral referred pain, and pain-related symptoms may persist after the resolution of prostatitis. There is evidence showing that abnormal neural regulation is involved in the pathogenesis of CP related pain (3). Studies have shown that the pathogenesis of CP/CPPS in males and interstitial cystitis/bladder pain syndrome in females is related to inflammation, immunity, and neuropathology. Minimally invasive treatments (including spinal cord and peripheral nerve stimulation) may become promising for the treatment of refractory CP-related pain (3). Spinal microglia are important immune cells in the central nervous system (CNS), and they can cause pain by secreting inflammatory mediators in response to the activation of NOTCH signaling pathways (4). Our previous pilot study also reveals that NOTCH1 is involved in the transmission of signals in the glial cells, which mediates neuropathic pain and NOTCH1 is one of the key molecules regulating pain (unpublished data). However, the role of NOTCH1-mediated microglial activation in persistent pain related to CP remains

unclear. This study aimed to investigate whether NOTCH1 induces microglia in the L5-S2 segment of the spiral cord to secrete inflammatory cytokines and subsequently induce central pain sensitization in a rat model of CP-related pain. Our findings may be helpful to elucidate the role of neuroinflammation in the pathogenesis of CP-related pain.

Materials and Methods

Grouping. The rat model of CP-related pain was established according to a previously reported study (5, 6). Adult male specific pathogen-free rats (200±25 g) were purchased from the Animal Experiment Center of the Army Military Medical University (Chongqing, PR China), and randomly divided into a CP group (n=6 at each time point) and a control group (n=6 at each time point). This study was approved by the Ethics Committee of Guiqian International General Hospital (no. 2019-0160). All the animal procedures were conducted according to the Guidelines of the International Council for Laboratory Animal Science.

Establishment of CP-related pain model. Rats were intraperitoneally anesthetized with 1% pentobarbital sodium (0.3 ml/100 g bodyweight), and the prostate was exposed through a small middle incision (1 cm) in the lower abdomen. In the CP group, 10 μl complete Freund's adjuvant (CFA) (Sigma, St. Louis, MO, USA) was injected at both sides of the prostate. In the control group, an equal amount of normal saline was injected. Then, the wound was closed. Histological examination and assessment of pain-related behaviors were carried out, aiming to evaluate the successful establishment of a CP-related pain model.

Evaluation of pain-related behavior. At 0, 4, 12 and 24 days, pain related behaviors were examined. In brief, rats were placed in a plexiglass box (2 cm×12 cm×22 cm) with 3-mm bottom plate. Animals were allowed to acclimatize to the environment for 30 min, and then a thermal stimulator (12 V, 50 W) was used to stimulate the pelma of the rats over a 5-mm spot. The time to paw withdrawal was recorded as thermal withdrawal latency. Each stimulation was no longer than 30 s, and 30 s was recorded as the time to withdrawal if the time was longer than 30 s. The interval between making two measurements was at least 10 min. The examination was done five times per animal. The maximum and minimum values were removed, and the average was calculated from the remaining values.

Histological examination. At 0, 4, 12 and 24 days after CFA injection, rats (n=6 at each time point) were anesthetized by intraperitoneal injection of 1% pentobarbital sodium at 0.3 ml/100 g bodyweight, and then the heart was perfused with 200 ml of 0.9% normal saline and then with 300 ml of 0.01 mol/l phosphate buffer solution containing 4% paraformaldehyde at 48˚C. The left and right prostate tissues were collected after laparotomy, then fixed in 4% paraformaldehyde at 4˚C overnight, embedded in paraffin and sectioned for pathological examination. The sections were observed under a light microscope.

Intrathecal injection of NOTCH1 antagonist and inhibitor of microglial cells. An intrathecal catheter was placed as previously reported (7). In brief, in an independent experiment, rats were anesthetized by intraperitoneal injection of 1% pentobarbital sodium, and then the occipito-axial ligament was exposed after injection of CFA into the prostate. A PE-10 tube was carefully inserted into the subarachnoid space (about 6 cm in length). If cerebrospinal fluid was present in the tube, the outlet of this tube was sealed, followed by wound closure. The PE-10 tube was fixed to the skin. Animals were allowed to recover over the following 7 days. Animals without movement disorder after operation were used in subsequent experiments. Animals were divided into three groups (n=6 per group): i) NOTCH1 receptor antagonist group: animals were injected with tangeretin (Sigma) at 0.1 mmol/l; ii) Microglial inhibition group: animals were injected with minocycline (Sigma) at 5 mmol/l; iii) Control group: artificial cerebrospinal fluid (pH 5.5; Coolaber, Shanghai, PR China) of equal volume was injected (15 μl). Intrathecal injection was performed once daily for 5 consecutive days. After injection, the tube was flushed with 5 μl of artificial cerebrospinal fluid and the injection was completed within 5 min for all animals. The spinal cord tissues were collected 5 days later and processed for the determination of TNF-α and IL-1β by ELISA.

Determination of mRNA expression of NOTCH1 and IBA-1 by quantitative real time polymerase chain reaction. In the CP group, rats were decapitated after anesthesia at 0, 4, 12 and 24 days after CFA injection. The L5-S2 segments of the spinal cord were removed from the posterior horn on both sides. The spinal cord tissues were weighed and then processed for the extraction of total RNA. The purity of total RNA was determined by spectrophotometry at 260 nm and 280 nm (ratio of A260 to A280 at 1.8-2.0). Then 2 μg of total RNA was processed for reverse transcription into cDNA under following conditions: 70˚C for 5 min → 37˚C for 60 min → 70˚C for 10 min. Furthermore, 20 ng of cDNA was used for the detection of target genes by quantitative real time polymerase chain reaction under the following conditions: 50˚C for 30 min, 95˚C for 15 min, 94˚C for 15 s, 56˚C for 15 s, 72˚C for 30 s, for a total of 40 cycles. glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as the internal reference. A standard curve was drawn, and the expression of target genes was normalized to that of *GAPDH*. The primers used in the present study were as follows: *IBA-1*: TTGATCTGAATGGCAATGGA (forward) and CCTCCAATTA GGGCAACTCA (reverse); *NOTCH1*: CATCACCTGCCTGTTA GGAG (forward) and ACACATGGCAACATCTAACCC (reverse); *GAPDH*: TTTAACTCTGGTAAAGTGGATATTGTTG (forward) and ATTTCCATTGATGACAAGCTTCC (reverse).

Detection of TNF-α and IL-1β contents by enzyme-linked immunosorbent assay (ELISA). At 0, 4, 12 and 24 days after CFA injection, the spinal dorsal horn of L5-S2 segments was collected, and then lysed in 0.5 ml of lysis solution (50 mMTris, 150 mM NaCl, 1% Triton X 100, 0.5% sodium deoxycholate, 1 mM of phenylmethanesulfonyl fluoride (Shanghai Sangong, Shanghai, PR China), 0.1% sodium dodecyl sulfate, 10 mM sodium fluoride and 1 mM sodium sulfate) on ice. After homogenization on ice, centrifugation was performed at 1,200 g/min for 10 min. The supernatant was collected for the detection of IL-1β and TNF-α by ELISA [Rat IL-1 beta ELISA Kit (ab255730) and Rat TNF alpha ELISA Kit (ab236712); Shanghai Sangong].

Measurement of neuronal electrical excitability by patch clamp. At 0, 4, 12 h and 24 h, animals were sacrificed, and the L5-S2 segments were collected and sliced for the detection of neuronal

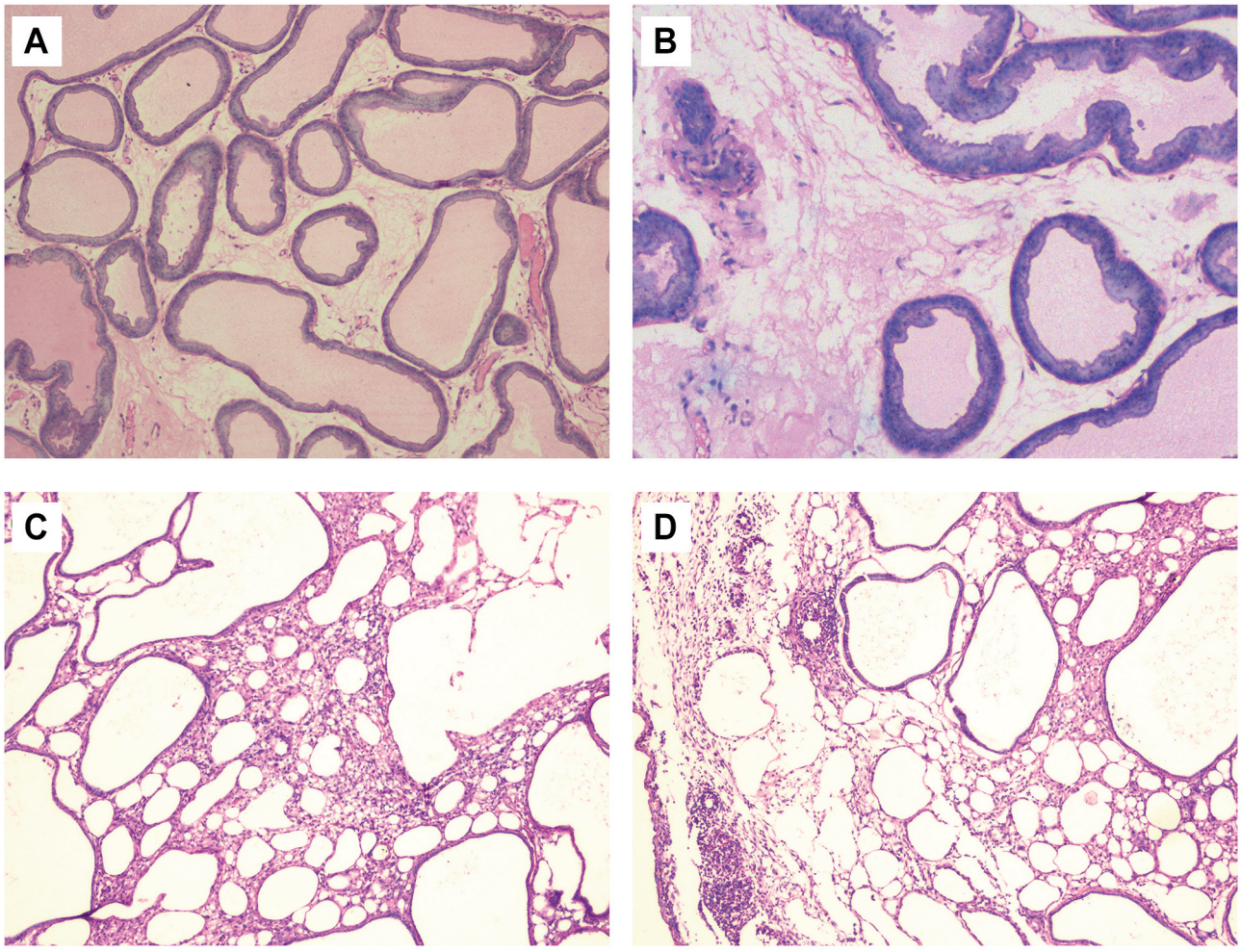

Figure 1. *Pathological examination of the prostate at 0 (A), 4 (B), 12 (C) and 24 (D) days in rats after induction of chronic prostatitis (hematoxylin and eosin: ×100).*

electrical excitability with the whole-cell patch clamp method. In brief, the spinal cord was quickly collected from animals after anesthesia, and L5-S2 slices (500 μm in thickness) were obtained with a microtome. These slices were incubated with artificial cerebrospinal fluid at a constant temperature. Then an electrode (5-8 MΩ) was used to clamp the superficial neurons of the dorsal horn and the electrophysiological characteristics of neurons were measured after the data stabilized. The whole-cell recording mode was used under Clampex sampling. A series of step currents (−50 – +140 pA; step=10 pA, duration=1,000 ms) were administered to induce electrical activity from hyperpolarization to depolarization with a current clamp (I=0), the voltage–current curve was obtained, and the evoked action potential (AP) was observed when the membrane potential exceeded the threshold. The basic parameters regarding electrical excitability of neurons (resting membrane potential, threshold, and amplitude of AP) were recorded. The spontaneous electrical activity of neurons was observed under the Gapfree continuous recording mode and compared with normal controls. Increased electrical excitability indicates enhanced sensitivity to central pain.

Statistical analysis. All data are expressed as the mean±standard deviation. Statistical Product and Service Solutions version 13.0 (IBM, Armonk, NY, USA) was used for the statistical analysis. Quantitative data are expressed as mean±standard deviation, and qualitative data are expressed as median. Comparisons of data with normal distribution were made among groups with one-way analysis of variance. A value of $p<0.05$ was considered statistically significant.

Results

Prostate pathology and pain-related behaviors. In the CP group, cell degeneration, necrosis and shedding were observed; a large number of lymphocytes and monocytes infiltrated the stroma, and some lymphocytes clustered in the prostate. In the control group, the prostate structure remained intact without obvious infiltration of inflammatory cells (Figure 1). This indicated that CFA caused prostatitis in the rats. In the CP group, the thermal withdrawal latency was

Table I. *Thermal withdrawal latency (s) in rats after induction of chronic prostatitis (CP) and in controls (n=6 at each point).*

	Time point (days)			
Group	0	4	12	24
Control	22.56±1.50	22.14±1.60	22.79±1.18	22.58±1.37
CP	22.27±1.65	14.61±0.90**	12.29±1.20**	14.01±1.36**

Significantly different at: **$p<0.01$ *vs*. Control.

Table II. *mRNA expression of NOTCH receptor 1 (NOTCH1) and ionized calcium binding adapter molecule 1 (IBA-1) in L5-S2 spinal cord segments in control rats and after induction of chronic prostatitis (CP) (n=6 at each point).*

Variable	Time point (days)	Control group	CP group
NOTCH1	0	0.330±0.036	0.329±0.039
	4	0.391±0.034	0.695±0.028**▲▲
	12	0.371±0. 029	0.895±0. 035**▲▲#▼
	24	0.359±0. 032	0.635±0. 036**▲▲
IBA-1	0	0.407±0. 033	0.395±0. 035
	4	0.392±0.041	0.711±0. 034**▲▲
	12	0.399±0.039	0.897±0.029**▲▲#▼
	24	0.431±0.037	0.856±0.030**▲▲

Significantly different at: **$p<0.01$ *vs*. Control; ▲▲$p<0.01$ *vs*. 0 d; #$p<0.05$ *vs*. 4 d; ▼$p<0.05$ *vs*. 24 d.

Table III. *Levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in L5-S2 spinal cord segments in rats after induction of chronic prostatitis (CP) and in controls (n=6 at each point).*

Variable	Time point (days)	Control group	CP group
TNF-α (ng/ml)	0	0.446±0.041	0.451±0.037
	4	0.448±0.031	0.672±0.038**▲▲
	12	0.454±0.035	0.790±0.038**▲▲#▼
	24	0.439±0.029	0.772±0.034**▲▲
IL-1β (ng/ml)	0	0.362±0.028	0.347±0.028
	4	0.372±0.026	0.491±0.037**▲▲
	12	0.350±0.027	0.591±0.026**▲▲#
	24	0.362±0.017	0.555±0.025**▲▲

Significantly different at: **$p<0.01$ *vs*. Control; ▲▲$p<0.01$ *vs*. 0 d; #$p<0.05$ *vs*. 4 d; ▼$p<0.05$ *vs*. 24 d.

Table IV. *Levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in L5-S2 spinal cord segments after inhibition of NOTCH receptor signaling pathway using NOTCH receptor 1 inhibitor tangeretin or inhibition of microglial cells with minocycline.*

Variable	CSF	Tangeretin	Minocycline
IL-1β (ng/ml)	0.581±0.028	0.401±0.027**	0.492±0.035**
TNF-α (ng/ml)	0.671±0.023	0.381±0.033**	0.401±0.022**

CSF: Artificial cerebrospinal fluid. **Significantly different at $p<0.01$ *vs*. CSF.

significantly shortened as compared to the control group ($p<0.01$). This indicated hyperalgesia in the rats after CFA injection. The above findings suggest the successful establishment of CP (Table I).

mRNA expression of NOTCH1 and IBA-1. Results showed the mRNA expression of IBA-1 and NOTCH1 in the L5-S2 segments of CP group was significantly higher than in the control group at 4, 12 and 24 days after CFA injection ($p<0.01$). Moreover, it peaked at day 12. This indicate that the microglial cells in the L5-S2 segments of CP rats were activated, and this was accompanied by NOTCH1 activation (Table II).

TNF-α and IL-1β in L5-S2 spinal cord segments. TNF-α and IL-1β in L5-S2 spinal cord segments were determined by ELISA. The results showed TNF-α and IL-1β began to increase at 4 days, peaked at 12 days and began to decrease at 24 days. At all time points after baseline, the levels of TNF-α and IL-1β in the CP group were significantly higher than in the control group ($p<0.01$) (Table III). After intrathecal injection of NOTCH1 antagonist or microglial inhibitor, TNF-α and IL-1β levels in L5-S2 spinal cord segments were markedly lower than in the CP group ($p<0.01$) (Table IV).

Electrical excitability. The whole-cell patch clamp method was used to record electrical excitability. In the control group, the resting membrane potential was -54.08±4.10 mV (n=30); in the CP group, the mean resting membrane potential was −52.22±4.99 mV (n=28), −55.12±4.76 mV (n=39) and −54.20±4.67 mV (n=36) at 4, 12 and 24 days, respectively. The series resistance was 10.78±3.78 MΩ in the control group; in the CP group, it was 10.74±2.15 MΩ, 11.67±2.15 MΩ and 10.99±2.18 MΩ at 4, 12 and 24 days, respectively. The membrane capacity was 10.79±2.29 pF in the control group and was 11.63±3.15 pF, 12.12±3.07 pF and 12.10±3.11 pF in the CP group at 4, 12 and 24 days, respectively. The passive membrane electrophysiological characteristics of neurons remained unchanged after induction of CP ($p>0.05$).

In the current clamp-recording mode, step currents were administered to neurons to induce electric activity from hyperpolarization to depolarization, and then the AP was

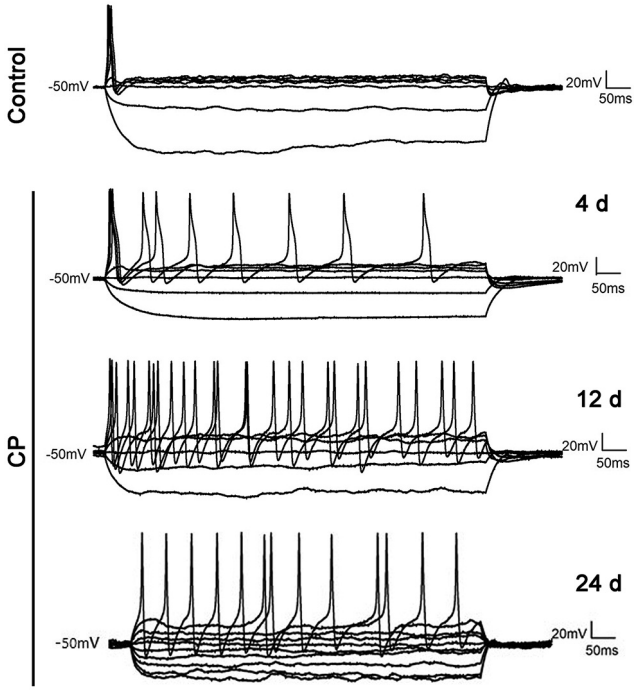

Figure 2. *Action potential characteristics of L5–S2 neurons after induction of chronic prostatitis (CP) in rats. A series of step currents (–50 to +140 pA, step=10 pA, duration=1,000 ms) were administered to induce electrical activity from hyperpolarization to depolarization with a current clamp (I=0), and the voltage–current curve was obtained, an evoked action potential (AP) was observed when the membrane potential exceeded the threshold. The resting membrane potential, threshold and amplitude of AP were recorded.*

Figure 3. *Spontaneous electrical activity of L5–S2 neurons 12 days after chronic prostatitis (CP) was induced in rats. At days 12 and 24, the fluctuation in electrical activity was evident in the CP group, significant fluctuation was observed in several neurons, and even spontaneous AP was generated at the threshold.*

recorded (Figure 2). The amplitude, frequency, and threshold of AP at different time points in both groups are shown (Table V). The results showed the amplitude and frequency of AP increased significantly, but the threshold reduced markedly compared to the control group ($p<0.05$), and spontaneous AP was observed (Figure 3).

In the current clamp mode, the membrane potential of neurons fluctuated autonomously when the clamp voltage was maintained at about −50 mV. The amplitude of membrane potential fluctuated slightly in the control group and CP group at 4 days. However, fluctuation was evident in the CP group at 12 and 24 days; significant fluctuation was observed in several neurons, and even spontaneous AP was generated at the threshold.

Discussion

A relatively recent epidemiological study showed that the incidence of prostatitis-related symptoms in men under 50 years of age was as high as 11.5%, with type IIIa/CPPS (type IIIb) accounting for about 90% of prostatitis in clinical practice (8). The etiology and pathogenesis of CP-related pain are still unclear. Long-term pain may cause severe physical and mental disorders in patients with CP. Currently, no effective treatment has been developed for CP-related pain, and the diagnosis and treatment of CP-related pain have become a challenge in the field of urology (9). Therefore, the investigation of pathogenesis of CP-related pain is extremely important for the development of therapeutic strategies for this disease.

Bryk *et al*. proposed the well-known UPOINT classification system for prostatitis, which provides a new way for the diagnosis and treatment of CP, and has been widely recognized and used. UPOINT classifies patients into six domains: Urinary, psychosocial, organ-specific, infectious, neurological/systemic and tenderness of skeletal muscles. As shown by this classification system, CP-related pain is often characterized by refractory perineal pain and discomfort to varying degrees, frequently accompanied by bladder and urethral dysfunction, which is the main reason for a hospital visit in these patients (9). Although some studies have been conducted to investigate the pathogenesis of CP-related pain and the therapies of CP-related pain have improved in recent years, the quality of life of patients with CP remains unchanged and they still display pain and urinary symptoms (10). Therefore, the pathogenesis of CP-related pain may not be confined to prostate infection, abnormal regulation of CNS may also be involved (11). CP-related pain often has two clinical characteristics: i) Pain is generalized. Pain is not confined to the prostate, it involves the areas innervated by the L5-S2 spinal cord segments which are related to bladder and urethral function. Spontaneous pain can occur in parts without primary lesions, and muscular dysfunction of the bladder and urethra may be present to varying degrees, suggesting the characteristics of visceral referred pain; ii) Pain is persistent. In clinical practice, some patients still present CP-related pain after the resolution of prostatitis. Therefore, CP-related pain may be a type of persistent visceral referred pain, and its

Table V. *Characteristics of neuronal action potential in L5-S2 spinal cord segments after induction of chronic prostatitis (CP) in rats and in controls.*

Group	Time point (days)	No. of measurements	Amplitude (mV)	Frequency (n)	Threshold (mV)
Control	25	93.67±8.50	1.99±0.08	−17.1±3.31	
CP	4	29	103.04±11.82*	7.98±1.32*	−22.41±3.04*
	12	39	115.87±12.61*	10.21±1.29*	−32.31±5.54*
	24	40	119.80±12.64*	9.55±1.58*	−30.30±5.15*

*Significantly different at $p<0.05$ *vs.* Control group.

pathogenesis is closely related to the changes of nerves in the spinal cord that innervate the prostate (12). In our previous study, a rat model of stable prostate pain was successfully established, and results showed the glial cells in the L5-S2 segments were activated in rats with CP-related pain, which was closely related to central hyperalgesia and had the involvement of visceral referred pain in CP (13, 14).

The dorsal horn of the spinal cord is an important center for pain modulation and integration. Study has shown that pain modulation is related to not only the neural network, but also to astrocytes and microglia (15). Pain signals can activate glial cells and neurons through a variety of inflammatory cytokines and miRNAs in exosomes, which is a key factor for the generation and maintenance of chronic neuropathic pain (16). Microglial cells are widely distributed in the CNS. Under physiological conditions, microglial cells are considered the 'quiescent' cells of the CNS. However, when the CNS is stimulated by trauma, ischemia, infection and other harmful stimuli, microglial cells may be immediately activated to produce pain modulators or pain-sensitizing substances, such as brain-derived neurotrophic factor, ILs, TNF and other inflammatory cytokines. These cytokines may increase the local synaptic plasticity and synaptic efficiency, and the elevated production of these factors can cause pain sensitization of neurons (17), which is involved in the regeneration of nociceptors after nerve injury. In animal models of nerve injury and pain, the production of inflammatory cytokines increases significantly in glial cells. Hypersensitivity in rats with pathological pain is inhibited after intrathecal injection of transcription factors or antagonists of inflammatory factors (18). In addition to the production of inflammatory factors, there is interaction between microglia and neurons. Microglia can affect the activity of neurons. Study has revealed that inhibiting the activation of microglial cells can compromise pain hypersensitivity (19). In a model of sciatic nerve inflammatory injury, intrathecal injection of minocycline (an inhibitor of microglial activation) inhibited mechanical hyperalgesia (20). In addition, the activation of glial cells has an involvement of specific signal pathways. Recently, the role of NOTCH in the activation of neurons and glial cells has attracted great attention in the field of pain. Inhibition of key signaling pathways may regulate the generation and tolerance of chronic neuropathic pain (21).

The NOTCH signaling pathway is highly conservative, can regulate cell differentiation and proliferation, and plays a key role in the regulation of embryonic development and cell fate. There are four NOTCH receptors (NOTCH 1-4) and multiple ligands in mammalian nervous systems: JAGGED1 and 2, and DELTA1, 3 and 4. These are single transmembrane proteins. The extracellular segments of NOTCH and its ligand have a repeat region of epidermal growth factor, which can be modified by glycosylation. NOTCH receptor also has several regions to keep the receptor inactivated in the absence of ligands. After the binding of receptor to ligand, the γ-secretase complex can cleave the transmembrane segment of NOTCH, then the intracellular segment of NOTCH is released into the cytoplasm, and the extracellular segment of NOTCH enters the 'signal sending cells' (ligand-expressing cells) through endocytosis together with the ligand. Ligands can also be cleaved by the γ-secretase, which then transduces signals into cells (22). There is evidence showing that NOTCH can activate inflammatory cells to produce inflammatory mediators, playing a role in pain modulation (23).

In 2006, Mizuguchi *et al.* found that the NOTCH signaling pathway was an arbiter of neuronal excitation or inhibition, and involved in pain sensitization, but the specific mechanism was and still is unclear (24). NOTCH-mediated pain sensitization may be related to the classic mechanism of chronic pathological pain reported by Woolf and Salter (25). The physiological response to pain is a normal response in which peripheral nociceptive signal is transmitted to the cerebral cortex to produce the sensation of pain. In the early stage of pathological pain, the intracellular signal transduction pathway is activated *via* phosphorylation of receptors and channels, which reflects the post-translational change. However, chronic neuropathic pain is characterized by the long-term and continuous alterations of receptors, ion channels and intracellular signal transduction, which involves the expression of neurotransmitters, synaptic connections, neuronal structure, and survival and reflects nuclear transcription and translation. The activation of signal pathways in turn affects nuclear transcription and translation, which is regarded as an important process related to changes of neural

plasticity. The NOTCH signaling pathway participates not only in the proliferation and differentiation of neural stem cells, but also in learning and memory related to neurons in the embryonic stage. In adult mammals, NOTCH expression increases significantly after nerve injury, and is involved in glial cell activation and neural remodeling; and inhibiting the NOTCH signaling pathway has been found to exert analgesic effect (26, 27). In recent years, increasing attention has been paid to the role of the NOTCH signaling pathway in the pathogenesis of pain. NOTCH1 signaling is also involved in the neural plasticity of adult animals (28). The activation of NOTCH1 signaling in the dorsal horn of spinal cord increases the excitability of neurons, while its inhibition inhibits the excitability of neurons (29). In addition, the activation of NOTCH1 signaling can promote the differentiation of neural stem cells into astrocytes and microglia, which is related to synaptic remodeling and nerve regeneration (30). In an animal model of peripheral nerve injury, the activation of NOTCH1 signaling also activated glial cells, suggesting its important role in the plasticity of the adult nervous system after injury (31, 32). Moreover, NOTCH1 signaling is also involved in a variety of inflammatory responses to injury, in which it may interact with other inflammatory pathways (33). The NOTCH1 signaling pathway also affects neuronal excitability as well as the regeneration and activation of glial cells. There is evidence showing that the NOTCH1 signaling pathway may also regulate the expression of membrane receptors, ion channels, and neurotransmitters, as well as synaptic plasticity (34, 35), but these mechanisms need to be further studied.

CP-related pain, as a common inflammatory visceral pain, has a high incidence and is often refractory to available treatments. It is very important to investigate the relationship and interaction among neuroimmune, peripheral and central mechanisms of pain, which is helpful for the elucidation of pathogenesis of CP-related pain and for the identification of targets for the development of pharmacotherapeutics.

In the present study, our results showed NOTCH1 expression and IBA-1 expression increased in the spinal cord of rats with CP-related pain; inhibiting microglial activation and NOTCH1 signaling pathway significantly reduced the production of inflammatory cytokines, thereby relieving CP-related pain.

Conclusion

Our study confirmed the abnormal neurological regulation at the L5-S2 level of the spinal cord in CP-related pain, and the activation of microglial cells in the spinal cord plays an important role in the occurrence and maintenance of pain related to the activation of NOTCH1 signaling pathway in the spinal cord. Based on our findings, we speculate that the activation of the NOTCH1 signaling pathway may induce the activation of microglial cells, leading to the secretion of a large amount of inflammatory cytokines, which is involved in the transmission of nociceptive signals and pain modulation and plays a key role in central hyperalgesia.

Conflicts of Interest

The Authors declare that they have no competing interests.

Authors' Contributions

Conceptualization: Heng Zhang and Chuanhua Zhong. Methodology and experimentation: Heng Zhang, Ruifen Gu and Jisheng Luo. Software and formal analysis: Chuanhua Zhong and Heng Zhang. Data curation: Heng Zhang. Writing – original draft preparation: Heng Zhang. Writing – review and editing: Jinhong Pan. Project administration: Jinhong Pan. Funding acquisition: Jinhong Pan.

References

1 Magistro G, Wagenlehner FM, Grabe M, Weidner W, Stief CG, Nickel JC: Contemporary management of chronic prostatitis/chronic pelvic pain syndrome. Eur Urol 69(2): 286-297, 2016. DOI: 10.1016/j.eururo.2015.08.061

2 Wang J, Cao B, Lin S, Wang Y, Zhang Q, Jiang Y, Li W, Zhu Y: A bibliometric analysis of urologic chronic pelvic pain syndrome from 2000 to 2022. J Pain Res 16: 1225-1241, 2023. DOI: 10.2147/JPR.S396009

3 Adamian L, Urits I, Orhurhu V, Hoyt D, Driessen R, Freeman JA, Kaye AD, Kaye RJ, Garcia AJ, Cornett EM, Viswanath O: A comprehensive review of the diagnosis, treatment, and management of urologic chronic pelvic pain syndrome. Curr Pain Headache Rep 24(6): 27, 2020. DOI: 10.1007/s11916-020-00857-9

4 Chen J, Ding H, Liu B, Zhou X, Zhou X, Lin Z, Yang F, Zhan H, Xiao H: Notch1 signaling contributes to mechanical allodynia associated with cyclophosphamide-induced cystitis by promoting microglia activation and neuroinflammation. Mediators Inflamm 2021: 1791222, 2021. DOI: 10.1155/2021/1791222

5 Butler SH, Godefroy F, Besson J, Weil-Fugazza J: A limited arthritic model for chronic pain studies in the rat. Pain 48(1): 73-81, 1992. DOI: 10.1016/0304-3959(92)90133-V

6 Nackley AG, Suplita RL 2nd, Hohmann AG: A peripheral cannabinoid mechanism suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. Neuroscience 117(3): 659-670, 2003. DOI: 10.1016/s0306-4522(02)00870-9

7 Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. Physiol Behav 17(6): 1031-1036, 1976. DOI: 10.1016/0031-9384(76)90029-9

8 Clemens JQ, Mullins C, Ackerman AL, Bavendam T, van Bokhoven A, Ellingson BM, Harte SE, Kutch JJ, Lai HH, Martucci KT, Moldwin R, Naliboff BD, Pontari MA, Sutcliffe S, Landis JR, MAPP Research Network Study Group: Urologic chronic pelvic pain syndrome: insights from the MAPP Research Network. Nat Rev Urol 16(3): 187-200, 2019. DOI: 10.1038/s41585-018-0135-5

9 Bryk DJ, Shoskes DA: Using the UPOINT system to manage men with chronic pelvic pain syndrome. Arab J Urol 19(3): 387-393, 2021. DOI: 10.1080/2090598X.2021.1955546

10 Aktar N, Moudud A, Chen T, Gao X, Min H, Tang M, Zhou X: Recent advances in pharmacological interventions of chronic prostatitis/chronic pelvic pain syndrome. Curr Pharm Des 27(25): 2861-2871, 2021. DOI: 10.2174/1381612827666210322125054

11 Deng GC, Lu M, Zhao YY, Yuan Y, Chen G: Activated spinal astrocytes contribute to the later phase of carrageenan-induced prostatitis pain. J Neuroinflammation 16(1): 189, 2019. DOI: 10.1186/s12974-019-1584-3

12 Antolak SJ, Antolak CM: Chronic pelvic pain: Neurogenic or non-neurogenic? Warm detection threshold testing supports a diagnosis of pudendal neuropathy. Pain Physician 21(2): E125-E135, 2018.

13 Zhang H, Liu L, Yang Z, Pan J, Chen Z, Fang Q, Li W, Li L, Lu G, Zhou Z: P2X7 receptor mediates activation of microglial cells in prostate of chemically irritated rats. Int Braz J Urol 39(2): 276-285, 2013. DOI: 10.1590/S1677-5538.IBJU.2013.02.17

14 Zhang H, Liu L, Lu G, Chen Z, Fang Q, Yang Z, Li L, Li W, Song B, Zhou Z: Chemical irritation of the prostate sensitizes P_2X_3 receptor-mediated responses in rat dorsal root ganglion neurons. Neurourol Urodyn 30(4): 612-618, 2011. DOI: 10.1002/nau.21060

15 Miranpuri GS, Bali P, Nguyen J, Kim JJ, Modgil S, Mehra P, Buttar S, Brown G, Yutuc N, Singh H, Wood A, Singh J, Anand A: Role of microglia and astrocytes in spinal cord injury induced neuropathic pain. Ann Neurosci 28(3-4): 219-228, 2021. DOI: 10.1177/09727531211046367

16 Jean-Toussaint R, Lin Z, Tian Y, Gupta R, Pande R, Luo X, Hu H, Sacan A, Ajit SK: Therapeutic and prophylactic effects of macrophage-derived small extracellular vesicles in the attenuation of inflammatory pain. Brain Behav Immun 94: 210-224, 2021. DOI: 10.1016/j.bbi.2021.02.005

17 Dominguini D, Steckert AV, Michels M, Spies MB, Ritter C, Barichello T, Thompson J, Dal-Pizzol F: The effects of anaesthetics and sedatives on brain inflammation. Neurosci Biobehav Rev 127: 504-513, 2021. DOI: 10.1016/j.neubiorev.2021.05.009

18 Marcos JL, Galleguillos D, Pelissier T, Hernández A, Velásquez L, Villanueva L, Constandil L: Role of the spinal TrkB-NMDA receptor link in the BDNF-induced long-lasting mechanical hyperalgesia in the rat: A behavioural study. Eur J Pain 21(10): 1688-1696, 2017. DOI: 10.1002/ejp.1075

19 Inoue K, Tsuda M: Microglia in neuropathic pain: cellular and molecular mechanisms and therapeutic potential. Nat Rev Neurosci 19(3): 138-152, 2018. DOI: 10.1038/nrn.2018.2

20 Chen G, Luo X, Qadri MY, Berta T, Ji RR: Sex-dependent glial signaling in pathological pain: Distinct roles of spinal microglia and astrocytes. Neurosci Bull 34(1): 98-108, 2018. DOI: 10.1007/s12264-017-0145-y

21 Sanna MD, Borgonetti V, Galeotti N: μ opioid receptor-triggered Notch-1 activation contributes to morphine tolerance: Role of neuron–glia communication. Mol Neurobiol 57(1): 331-345, 2020. DOI: 10.1007/s12035-019-01706-6

22 Kopan R, Ilagan MX: The canonical Notch signaling pathway: unfolding the activation mechanism. Cell 137(2): 216-233, 2009. DOI: 10.1016/j.cell.2009.03.045

23 Zhang Y, Wang T, Wu S, Tang L, Wang J, Yang J, Yao S, Zhang Y: Notch signaling pathway: a new target for neuropathic pain therapy. J Headache Pain 24(1): 87, 2023. DOI: 10.1186/s10194-023-01616-y

24 Mizuguchi R, Kriks S, Cordes R, Gossler A, Ma Q, Goulding M: Ascl1 and Gsh1/2 control inhibitory and excitatory cell fate in spinal sensory interneurons. Nat Neurosci 9(6): 770-778, 2006. DOI: 10.1038/nn1706

25 Woolf CJ, Salter MW: Neuronal plasticity: Increasing the gain in pain. Science 288(5472): 1765-1768, 2000. DOI: 10.1126/science.288.5472.1765

26 Jin G, Hong L, Liu H, Yue R, Shen Z, Yang J, Xu Y, Huang H, Li Y, Xiong B, Su Y, Yu C: Koumine modulates spinal microglial M1 polarization and the inflammatory response through the Notch-RBP-Jκ signaling pathway, ameliorating diabetic neuropathic pain in rats. Phytomedicine 90: 153640, 2021. DOI: 10.1016/j.phymed.2021.153640

27 Miller SR, Benito C, Mirsky R, Jessen KR, Baker CVH: Neural crest Notch/Rbpj signaling regulates olfactory gliogenesis and neuronal migration. Genesis 56(6-7): e23215, 2018. DOI: 10.1002/dvg.23215

28 Alberi L, Hoey SE, Brai E, Scotti AL, Marathe S: Notch signaling in the brain: In good and bad times. Ageing Res Rev 12(3): 801-814, 2013. DOI: 10.1016/j.arr.2013.03.004

29 Li K, Lu M, Cui M, Wang X, Zheng Y: The Notch pathway regulates autophagy after hypoxic–ischemic injury and affects synaptic plasticity. Brain Struct Funct 228(3-4): 985-996, 2023. DOI: 10.1007/s00429-023-02639-6

30 Zhang YH, Wang T, Li YF, Deng YN, Shen FG: Roles of the Notch signaling pathway and microglia in autism. Behav Brain Res 437: 114131, 2023. DOI: 10.1016/j.bbr.2022.114131

31 Duan H, Shen F, Li L, Tu Z, Chen P, Chen P, Wang Z, Liang W, Wang Y: Activation of the Notch signaling pathway in the anterior cingulate cortex is involved in the pathological process of neuropathic pain. Pain 162(1): 263-274, 2021. DOI: 10.1097/j.pain.0000000000002014

32 Al-Massri KF, Ahmed LA, El-Abhar HS: Pregabalin and lacosamide ameliorate paclitaxel-induced peripheral neuropathy *via* inhibition of JAK/STAT signaling pathway and Notch-1 receptor. Neurochem Int 120: 164-171, 2018. DOI: 10.1016/j.neuint.2018.08.007

33 Lee S, Kim SK, Park H, Lee YJ, Park SH, Lee KJ, Lee DG, Kang H, Kim JE: Contribution of autophagy-Notch1-mediated NLRP3 inflammasome activation to chronic inflammation and fibrosis in keloid fibroblasts. Int J Mol Sci 21(21): 8050, 2020. DOI: 10.3390/ijms21218050

34 Mohammed H, Rimondini L, Rocchetti V: Molecular basis of trigeminal nerve disorders and healing. Eur Rev Med Pharmacol Sci 22(17): 5755-5764, 2018. DOI: 10.26355/eurrev_201809_15844

35 Ghareghani M, Zibara K, Sadeghi H, Dokoohaki S, Sadeghi H, Aryanpour R, Ghanbari A: Fluvoxamine stimulates oligodendrogenesis of cultured neural stem cells and attenuates inflammation and demyelination in an animal model of multiple sclerosis. Sci Rep 7(1): 4923, 2017. DOI: 10.1038/s41598-017-04968-z

Received August 18, 2023
Revised December 9, 2023
Accepted December 22, 2023