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Original Research Article

Pregabalin alleviates postherpetic neuralgia by downregulating spinal TRPV1 channel protein

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Abstract

Purpose: *To determine the mechanism involved in pregabalin-induced alleviation of postherpetic neuralgia in a rat model.*
Methods: *Ninety-sixty healthy Sprague-Dawley (SD) rats were assigned to sham, model and pregabalin groups (32 rats per group). A model of postherpetic neuralgia (PN) was established. The expressions of IL-1β and TNF-α in spinal cord tissue were determined 7 days after administration of treatments. The proportions of fluorescence areas in astrocytes in the dorsal horn, prefrontal lobe and hippocampus, and level of spinal cord TRPV1 channel protein in each group were evaluated.*
Results: *Relative to model rats, IL-1β and TNF-α in spinal cord of pregabalin rats were significantly reduced ($p < 0.05$). The areas of fluorescence in astrocytes in dorsal horn of spinal cord, prefrontal lobe and hippocampus of model group were significantly increased, relative to sham, but were decreased in rats in pregabalin group ($p < 0.05$).*
Conclusion: *Pregabalin significantly alleviates postherpetic neuralgia via mechanisms which may be related to the inflammatory response of spinal dorsal horn and downregulation of TRPV1 channel protein expression. This finding may be useful in developing new drugs for alleviating postherpetic neuralgia.*

Keywords: *Pregabalin, Spinal TRPV1 channel protein, Herpes zoster, Neuralgia, Mechanism*

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INTRODUCTION

Herpes zoster is an acute skin disease caused by herpes zoster virus. In the elderly, herpes zoster neuralgia is the most common complication of herpes zoster, and it manifests as a burning, tearing or knife-cut pain which results in difficulty in eating, decline in quality of life decline, and anxiety or depression [1]. The pathogenesis of postherpetic neuralgia is still not clearly understood. However, based on the pathological changes it induces in the nervous system, the disease is thought to be a type of neuropathic pain. Therefore, it is of great significance to study the pathogenesis of the disease and provide relevant scientific evidence for its therapy. Studies have found that the outcome of treatment for herpes zoster is closely

related to the time of onset: the shorter the duration of the disease, the earlier the treatment time and the better the treatment effect [2].

At present, the clinical treatment of postherpetic neuralgia involves the use of nucleoside analogs, antidepressants and local anesthetics. Although these strategies have resulted in some measure of efficacy, they are associated with toxic side effects [3]. Pregabalin, a new anticonvulsant and a first-line drug approved by the US Food and Drug Administration for the treatment of neuropathic pain, relieves pain, regulates sleep function, combats anxiety, and inhibits neuronal excitability [4]. However, the mechanism involved in its effect on postherpetic neuralgia is still unclear. In this study, the effect of pregabalin on a rat model of postherpetic neuralgia, and the mechanism involved, were investigated.

EXPERIMENTAL

Animals

Ninety-six healthy male SD rats aged 6 weeks, with mean body weight of 228 ± 20 g, were obtained from Chongqing Best Biological Technology Co. Ltd [production license SCXK (Chongqing) 2018-0004, SYXK (Chongqing) 2018-0004]. The rats were maintained at a laboratory at temperature of 23 ± 1 ^{o}C and humidity of 50 ± 10 % under 12-h light/12-h dark photoperiod, and were allowed *ad libitum* access to feed and water (adaptive feeding) for 7 days prior to commencement of the study. This study received approval from the Animal Ethics Committee of University of South China (approval no. 20200182), and was conducted according to "Principles of Laboratory Animal Care" [5].

Equipment and reagents

The major instruments and reagents used, and their suppliers were: constant temperature water bath (Tianjin Hengao Science and Technology Development Co. Ltd, model: HWT-20B); ultra-low temperature refrigerator (Hangzhou Aipu Instrument Equipment Co. Ltd, model: AP-60-60LA); electronic balance (Jinan Noble Laboratory Equipment Co. Ltd, model: EX225D); phosphate buffer (Beijing Wanjia Shouhua Biological Technology Co. Ltd); enzyme linked immunoassay kit (Mercak Biotech Ltd); phosphate buffer (Shanghai Yuanye Biotechnology Co. Ltd), and pregabalin (Chongqing Saiwei Pharmaceutical Co. Ltd, production batch number: 20190073, specification: 75 mg ×10 tablets).

Study design

The rats were assigned to sham, PN model and pregabalin groups (n = 32 each). A rat model of postherpetic neuralgia was established viz: resinous toxin (40 μg/mL) solution was prepared by dissolving 1 mg of the toxin in a mixture of 2.5 mL of 10 % Tween 80, 2.5 mL of 10 % ethanol and 20 mL of 80 % normal saline. Model and pregabalin rats received intraperitoneal injection of resinous toxin solution at a dose of 200 μg/kg, while rats in the sham group were given injection saline in place of toxin. The pregabalin group was given pregabalin (0.03 g/kg) for one week following establishment of the model. The injection was given once daily for 7 days.

Parameters evaluated

Thermal and mechanical pain thresholds (TMPTs) of each group were measured using thermal pain measuring instrument and electronic pain measuring instrument, respectively. In the determination of mechanical pain threshold, the rats were put on an elevated metal cage in a quiet environment with constant temperature and humidity. The plantar surface of each rats was stimulated with filaments from a vertical angle. The stimulus was gradually increased until the rat appeared to lift and lick their feet.

The environment for measuring thermal pain threshold was the same as that for mechanical pain threshold. When the rats were quiet, they were placed on the stimulation hot plate. When the rats showed positive reactions such as foot lifting and licking, the times were recorded and the critical value was set at 30 sec. A total of 4 measurements were made, and the mean value of multiple measurements was recorded. The interval between tests was 15 min. Changes in mechanical pain threshold and thermal pain threshold were measured before establishment of the PN model, 1 week after PN model established, and 1 week after treatment.

One week after treatment, 8 rats were selected from each group. The spinal dorsal horn tissue levels of IL-1β and TNF-α of rats were determined. The tissue was homogenized and centrifuged, and an appropriate amount of chloroform was added to the supernatant. The supernatant was centrifuged, and total RNA was precipitated by addition of appropriate amounts of isopropanol and 75 % ethanol. The RNA was reverse-transcribed to cDNA in reaction system at 42 ^{o}C. One week after administration, spinal dorsal horn and brain tissues were taken from 12 rats in each group. The fluorescence areas of

astrocytes in spinal dorsal horn, prefrontal lobe and hippocampus were determined using immunofluorescence method. The frozen tissues were processed into 30-μm thick sections, washed with phosphate buffer solution, sealed with BSA solution, and subjected to overnight incubation at 4 °C with 1° immunoglobulins, prior to incubation with secondary immunoglobulin at 37 °C for 60 min, natural drying, sealing and examination under a fluorescence microscope.

The expression levels of transient receptor potential vanilloid type1 (TRPV1) channel protein were determined with immunohistochemistry and western blot assays. Immunohistochemistry, the spinal cord tissues were processed into paraffin sections which were dehydrated and dewaxed. Antigen recovery was done by incubation of the paraffin sections in sodium citrate antigen-repairing solution. Then, the sections were sealed with goat serum, prior to incubation overnight at 4 °C with primary antibody for TRPV1, rinsing with phosphate buffer, and incubation with secondary antibody at 37 °C for 30 min. Thereafter, the sections were stained with DAB and counterstained with hematoxylin, followed by dehydration, clearing and sealing. Finally, the stained sections were examined under a microscope. For Western blotting, total protein was extracted from spinal cord tissue by homogenization in ice-cold lysis buffer, and the homogenate was incubated for 30 min and centrifuged, and lysate protein content was measured with BCA procedure. Then, the proteins were resolved with SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes. The membranes were subjected to overnight incubation at 4 °C with 1° antibody for TRPV1, prior to treatment with secondary antibody linked to HRP at room temperature for 1 h. The bands were visualized with SignalFire™ Plus ECL system, while Image J software (vers. 2) was employed for quantification.

Statistics

Data are presented as mean ± standard deviation (SD). Comparison amongst multiple groups was done with univariate mean, while two-group comparison was done with independent sample *t*-test. Statistical evaluations were done with SPSS 24.0 software. Statistical significance was assumed at $p < 0.05$.

RESULTS

Mechanical pain thresholds and thermal pain thresholds (TMPTs)

Before modeling, there were no significant differences between TMPTs amongst the three groups ($p > 0.05$). However, relative to sham group, mechanical pain threshold in model rats was markedly reduced 1 week after establishing the PN model and 1 week after treatment, while the thermal pain threshold was significantly increased ($p < 0.05$). Compared with model group, mechanical pain threshold was significantly increased, while thermal pain threshold was significantly decreased 1 week after treatment ($p < 0.05$). These results are presented in Table 1.

Levels of TNF-α and IL-1β in rat spinal cord tissue

The expression levels of these cytokines in spinal cord of model rats were significantly increased, relative to the corresponding levels in the sham operation group. However, their expression levels in spinal cord on pregabalin-treated rats were significantly decreased ($p < 0.05$). These results are shown in Table 2.

Fluorescent areas of astrocytes in rat spinal dorsal horn, prefrontal lobe and hippocampus

Compared with sham group, the percentage of fluorescent areas in astrocytes in dorsal horn of spinal cord, prefrontal lobe and hippocampus of model group were significantly increased, but were markedly decreased in rats in pregabalin group ($p < 0.05$). These results are presented in Figure 1 and Table 3.

Table 1: Comparison of TMPTs amongst the groups

Group	Sham	Model	Pregabalin	*F*	*P*-value
Mechanical pain thresholds (g)					
Before PN model	46.58±2.33	46.79±1.52	45.87±2.33	2.91	0.060
1 week after PN model	46.28±2.83	8.79±1.82[a]	9.51±2.07[a]	2827.65	<0.001
1 week after treatment	45.17±2.24	9.31±1.79[a]	24.26±2.37[ab]	2250.71	<0.001
Thermal pain threshold (s)					
After PN model	7.28±1.57	7.24±0.83	7.86±1.32	2.36	0.100
1 week after PN model	7.38±1.68	24.46±2.34[a]	24.36±1.82[a]	799.36	<0.001
1 week after treatment	7.46±0.81	25.74±2.13[a]	12.40±1.25[ab]	1270.71	<0.001

Data are mean ± SD. [a, b]*P* < 0.05, vs sham ([a]); vs model ([b])

Table 2: Levels of IL-1β and TNF-α in the pulp tissue of rats (mean ± SD)

Group	IL-1β	TNF-α
Sham	0.98±0.04	1.00±0.01
Model	1.54±0.22 [a]	1.66±0.35 [a]
Pregabalin	1.21±0.26 [ab]	1.27±0.21 [ab]
F	16.17	15.85
P-value	<0.001	<0.001

[a, b]*P* < 0.05, vs sham ([a]); vs model ([b])

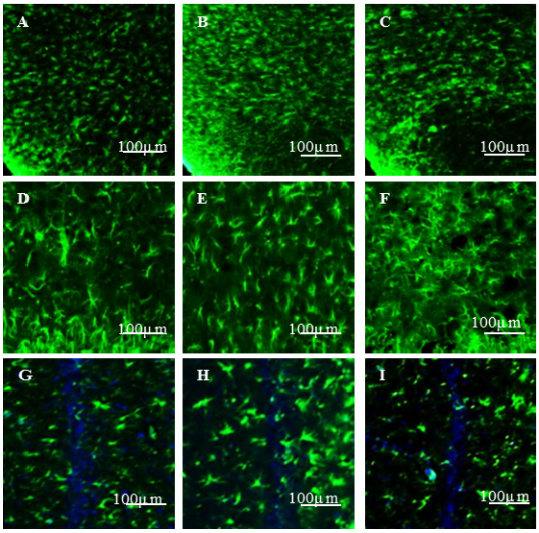

Figure 1: Relative fluorescence areas of astrocytes in spinal dorsal horn, prefrontal lobe and hippocampus of rats in each group. A-C: Spinal dorsal horn tissues of sham group (A), PN model rats (B) and pregabalin-treated rats (C). D -F: Prefrontal tissues of sham group (D), model group (E), and pregabalin group (F). G-I: Hippocampal CA1 tissues of sham group (G), model group (H) and pregabalin group (I)

Table 3: Fluorescent areas in astrocytes in spinal dorsal horn, prefrontal lobe and hippocampus of rats (%, n = 12)

Group	Spinal cord	Prefrontal lobe	CA1 of hippocampus
Sham operation group	9.68±0.92	12.24±1.32	6.55±0.81
Model group	16.85±0.79[a]	17.45±0.57[a]	12.41±1.82[a]
Pregabalin group	11.43±1.32[ab]	14.65±0.79[ab]	8.87±0.57[ab]
F	156.58	90.94	73.02
P-value	<0.001	<0.001	<0.001

Values are expressed as mean ± SD. [a]*P* < 0.05, compared with the sham operation group; [b]*p* < 0.05, compared with the model group

Protein expression levels of TRPV1 channel in rat spinal cord tissue

As shown in Figures 2 and 3 and in Table 4, relative to sham operation group, TRPV1 channel protein in the spinal cord of model rats was markedly up-regulated. However, the corresponding TRPV1 channel protein level in spinal cord of pregabalin-exposed rats was markedly decreased, relative to PN model group ($p < 0.05$).

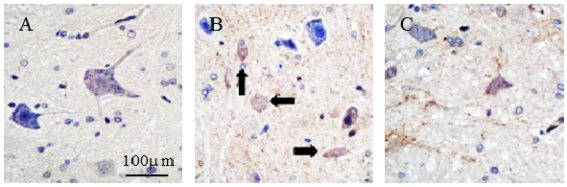

Figure 2: Expression levels of TRPV1 channel protein in rat spinal cord tissue. A: sham operation group, B: model group, C: pregabalin group

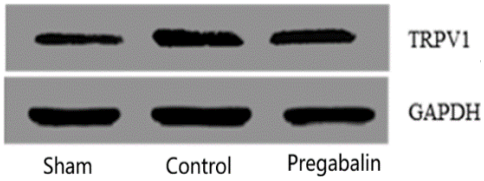

Figure 3: Expression level of TRPV1 channel protein in rat spinal cord tissue. [a, b]*P* < 0.05, vs sham operation rats ([a]); vs model rats ([b])

Table 4: Expression levels of TRPV1 channel protein in rat spinal cord tissue (mean ± SD)

Group	TRPV1
Sham	0.13±0.02
Model	0.19±0.03[a]
Pregabalin	0.16±0.02[ab]
F	19.06
P-value	<0.001

[a, b]*P* < 0.05, vs sham ([a]); vs model ([b])

DISCUSSION

Postherpetic neuralgia is a neuropathic pain caused by herpes zoster virus as a result of sensory nervous system injury, and it manifests mainly as persistent, paroxysmal, spontaneous knife pain or hyperalgesia. The pathogenesis of this disease is still poorly understood. However, it is believed that it may be closely related to age, regulation of expressions of related genes, and changes in immune function [6]. Age is the most important risk factor for postherpetic neuralgia. Statistics have shown that more than 13 % of herpes zoster patients aged over 55 years develop postherpetic neuralgia, the incidence of which increases with age, with negative impact on the physical and mental health of patients [7].

At present, the clinical treatment of postherpetic neuralgia uses mainly non-steroidal anti-inflammatory drugs and opioids which are aimed

at pain relief and improvement of quality of life, but long-term use of these drugs results in high degree of toxic and side effects [8]. Therefore, it will be of great significance to identify new drugs with lower adverse reactions and higher efficacy. Being an anti-epileptic drug, pregabalin selectively binds to α2-δ subunits of calcium ion channels, reduces the influx of calcium ions and inhibits the release of excitatory neurotransmitters, thereby producing analgesic effects. Moreover, its effect is faster and its safety is higher than those of other drugs [9]. Bihong *et al* found that after pregabalin treatment, patients with postherpetic neuralgia had significantly reduced pain scores and improved sleep quality [10]. In this study, the mechanism involved in pregabalin-induced mitigation of postherpetic nerve pain was determined and analyzed.

While pain is an unpleasant feeling or emotional experience related to actual or potential tissue damage, and an important pathophysiological reflex, neuropathologic pain lasts for a long time and seriously affects the life of the patient [11]. In this study, the TMPTs of each group of rats were determined. The results showed that pregabalin significantly relieved abnormal mechanical pain and thermal pain in rats with postherpetic nerve pain. This is in agreement with findings in studies which reported that pregabalin inhibited the excitability of cell membranes of nociceptive sensory neurons of the central nervous system, suppressed the transmission between synapses, and regulated the plasticity of associated neurons [12].

The spinal cord and brain play important roles in the pathogenesis of neuropathic pain, being vital control centers of the body [13]. Studies have shown that the activation of astrocytes promotes the release of TNF-α and other inflammatory factors, thereby aggravating the occurrence of neuropathic pain [14]. This investigation has demonstrated that pregabalin inhibited the activation of astrocytes in spinal dorsal horn, prefrontal lobe and hippocampus, and suppressed inflammatory response in spinal dorsal horn.

The transient receptor potential vanilloid type1 (TRPV1) is an important nociceptor molecule found in unmyelinated class C fibers and in some Aδ fibers in the dorsal root ganglion; it is closely related to a variety of physiological and pathological processes [15]. A variety of inflammatory substances or mediators sensitize TRPV1, leading to release of substance P, thereby producing pain sensation. The results of this study showed that TRPV1 level in the spinal cord of model rats was markedly increased [16]. This may be due to formation of local inflammation in the rat model of postherpetic nerve pain which resulted in an acidic environment which activated TRPV1 and enhanced pain generation, leading to occurrence of hyperalgesia [17-19]. However, pregabalin significantly down-regulated the expression of TRPV1.

CONCLUSION

This study has demonstrated that pregabalin significantly alleviates postherpetic nerve pain via a mechanism which may be related to inflammatory responses in spinal dorsal horn tissue, and downregulation of the expression of TRPV1 channel protein. This finding may be useful in developing new drugs for alleviating postherpetic neuralgia.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Authors' contribution

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Cong Zou designed the study, supervised the data collection, and analyzed the data. Zhenping Xiao interpreted the data and prepared the manuscript for publication. Mengjun Liao, Yunwu He, Yonglin Li, Wuzhou Yang and Liping Jiang supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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