



# Correlation of p38MAPK, ERK1/2 and JNK expression in peripheral blood with the cytokines and pain mediators in patients with post-herpetic neuralgia

Shi-Min Wang<sup>1</sup>✉, Xiao-Bing Liu<sup>2</sup>

<sup>1</sup> Department of Dermatology, the Second Hospital of Hanbin District Ankang City Shaanxi Province, Ankang City, Shaanxi Province, 725000

<sup>2</sup> Department of Dermatology, Ankang Central Hospital of Shaanxi Province, Ankang City, Shaanxi Province, 725000

## ARTICLE INFO

### Article history:

Received 8 Sep 2017  
Received in revised form 12 Sep 2017  
Accepted 19 Sep 2017  
Available online 28 Sep 2017

### Keywords:

Post-herpetic neuralgia  
Mitogen-activated protein kinase  
Cytokine  
Pain mediator

## ABSTRACT

**Objective:** To study the correlation of p38MAPK, ERK1/2 and JNK expression in peripheral blood with the cytokines and pain mediators in patients with post-herpetic neuralgia (PHN). **Methods:** Patients who were diagnosed with herpes zoster for the first time in the Second Hospital of Hanbin District Ankang City between March 2015 and April 2016 were selected and divided into the PHN group who were combined with post-herpetic neuralgia and the pure HZ group who were without neuralgia, and the healthy volunteers who received physical examination in the Second Hospital of Hanbin District Ankang City during the same period were selected as control group. The peripheral blood was collected to determine the expression of MAPK signaling molecules, and serum was collected to determine the contents of cytokines and pain mediators. **Results:** p38MAPK, ERK1/2 and JNK mRNA expression in peripheral blood as well as 5-HT, CGRP, SP, NSE, S100B, IFN- $\gamma$ , TNF- $\alpha$  and IL-17 levels in serum of PHN group and HZ group were significantly higher than those of control group while  $\beta$ -EP levels were significantly lower than that of control group; p38MAPK, ERK1/2 and JNK mRNA expression in peripheral blood as well as 5-HT, CGRP, SP, NSE, S100B, IFN- $\gamma$ , TNF- $\alpha$  and IL-17 levels in serum of PHN group were significantly higher than those of HZ group while  $\beta$ -EP level was significantly lower than that of HZ group. p38MAPK, ERK1/2 and JNK expression in peripheral blood of PHN patients were positively correlated with CGRP, 5-HT, SP, NSE, S100B, IFN- $\gamma$ , TNF- $\alpha$  and IL-17 levels in serum, and negatively correlated with  $\beta$ -EP level in serum. **Conclusion:** The high expression of p38MAPK, ERK1/2 and JNK in peripheral blood of PHN patients can increase the secretion of cytokines and algogenic mediators and decrease the secretion of analgesic mediators.

## 1. Introduction

Post-herpetic neuralgia (PHN) is the most common complication and sequelae of herpes zoster, it specifically refers to the persistent local neuropathic pain in affected part after the healing of local skin lesion caused by herpes zoster virus infection, lasts long and is quite difficult to handle, and the effect of conventional analgesic treatment of PHN is poor[1,2]. At present, the pathogenesis of PHN

has not been completely clear, inflammatory damage of neurons caused by herpes zoster virus infection is an important pathological feature of PHN, and the parasecretion of a variety of cytokines can cause abnormal synthesis of pain mediators, thus resulting in the occurrence of neuropathic pain[3]. Mitogen-activated protein kinase (MAPK) is an important signaling pathway regulating the inflammation of neurons, and the p38MAPK, ERK1/3, JNK and other signaling molecules are all common MAPK signaling molecules. In the following studies, we specifically analyzed the correlation of p38MAPK, ERK1/2 and JNK expression in peripheral blood with the cytokines and pain mediators in patients with post-herpetic neuralgia.

✉Corresponding author: Shi-Min Wang, Department of Dermatology, the Second Hospital of Hanbin District Ankang City Shaanxi Province, Ankang City, Shaanxi Province, 725000.

Tel: 0915-3612022; 13909158231

Fund Project: Project of Ankang Science and Technology Bureau No: 20130527.

## 2. Research subjects and methods

### 2.1. General information of research subjects

Patients with herpes zoster who were treated in the Second Hospital of Hanbin District Ankang City between March 2015 and April 2016 were selected, and all patients were diagnosed with herpes zoster for the first time, did not receive antiviral therapy before inclusion, and accepted systemic herpes zoster treatment after inclusion; patients combined with central or peripheral nerve injury and those with mental illness were excluded. According to the VAS score after treatment, the enrolled patients with herpes zoster were divided into the PHN group who were combined with post-herpetic neuralgia and the pure HZ group who were without neuralgia. Healthy volunteers who received physical examination in the Second Hospital of Hanbin District Ankang City during the same period were selected as control group, and they were without history of herpes virus infection, central or peripheral nerve injury diseases or mental illness. There were 39 cases in PHN group, including 22 men and 17 women that were 38-59 years old; there were 45 cases in pure HZ group, including 25 men and 20 women that were 35-57 years old; there were 50 cases in control group, including 29 men and 21 women that were 33-60 years old. There was no significant difference in general data among the three groups ( $P>0.05$ ).

### 2.2 Research methods

#### 2.2.1 Clinical sample collecting

5-6 mL of peripheral blood was collected from PHN group and HZ group before treatment, and 5-6 mL of peripheral blood was collected from control group during physical examination. Peripheral blood samples were divided into two parts, one was joined by Ficoll separating medium and then centrifuged to separate peripheral blood mononuclear cells, the cells were washed with PBS, and the cell pellet was placed at  $-80\text{ }^{\circ}\text{C}$ ; the other was let stand and centrifuged to separate the upper clear serum, which was move into the new EP tube and stored at  $-80\text{ }^{\circ}\text{C}$ .

#### 2.2.2 Peripheral blood signaling molecule expression testing

The cell pellet of peripheral blood mononuclear cells was taken and added in Trizol lysate to extract the total RNA in the cells, cDNA first-strand cDNA synthesis kit was used to synthesize the RNA into cDNA by reverse transcription, fluorescence quantitative PCR kit was used to amplify cDNA, the primers were for p38MAPK, ERK1/2, JNK and GAPDH, the Ct values of amplification curve was referred and the GAPDH was used as reference to calculate the p38 MAPK, ERK1/2 and JNK mRNA expression.

#### 2.2.3 Serum cytokine and pain mediator content testing

Serum samples were taken, and enzyme-linked immunosorbent assay kit was used to detect the contents of NSE, S100B, IFN- $\gamma$ , TNF- $\alpha$ , IL-17, 5-HT, CGRP, SP and  $\beta$ -EP.

**Table 2.**

Serum cytokine contents in three groups of subjects.

Groups	n	NSE	S100B	IFN- $\gamma$	TNF- $\alpha$	IL-17
PHN group	39	99.3 $\pm$ 10.2 <sup>#</sup>	127.6 $\pm$ 16.7 <sup>#</sup>	49.5 $\pm$ 6.7 <sup>#</sup>	18.6 $\pm$ 2.2 <sup>#</sup>	32.6 $\pm$ 5.2 <sup>#</sup>
HZ group	45	63.7 $\pm$ 8.1 <sup>*</sup>	70.5 $\pm$ 9.3 <sup>*</sup>	27.6 $\pm$ 3.8 <sup>*</sup>	11.2 $\pm$ 1.6 <sup>*</sup>	20.3 $\pm$ 2.9 <sup>*</sup>
Control group	50	35.2 $\pm$ 5.7	44.7 $\pm$ 6.2	14.2 $\pm$ 1.7	6.5 $\pm$ 0.9	11.7 $\pm$ 1.6

<sup>\*</sup>: compared with indexes of control group,  $P<0.05$ ; <sup>#</sup>: compared with indexes of HZ group,  $P<0.05$ .

### 2.3. Statistical methods

SPSS 19.0 software was used to input and analyze data, measurement data analysis among three groups was by variance analysis, the correlation between two data was by Pearson test and  $P<0.05$  indicated statistical significance in differences in test results.

## 3. Results

### 3.1 p38MAPK, ERK1/2 and JNK expression in peripheral blood

Analysis of signaling molecules p38MAPK, ERK1/2 and JNK expression in peripheral blood among the three groups of subjects was as follows: p38MAPK, ERK1/2 and JNK mRNA expression in peripheral blood of PHN group and HZ group were significantly higher than those of control group, and p38MAPK, ERK1/2 and JNK mRNA expression in peripheral blood of PHN group were significantly higher than those of HZ group. Differences in pair-wise comparison of p38MAPK, ERK1/2 and JNK expression in peripheral blood were statistically significant among the three groups of subjects ( $P<0.05$ ).

**Table 1.**

p38MAPK, ERK1/2 and JNK expression in peripheral blood of three groups of subjects.

Groups	n	p38 MAPK	ERK1/2	JNK
PHN group	39	2.94 $\pm$ 0.38 <sup>#</sup>	2.67 $\pm$ 0.34 <sup>#</sup>	3.28 $\pm$ 0.42 <sup>#</sup>
HZ group	45	1.88 $\pm$ 0.22 <sup>*</sup>	1.79 $\pm$ 0.20 <sup>*</sup>	2.05 $\pm$ 0.29 <sup>*</sup>
Control group	50	1.02 $\pm$ 0.13	0.98 $\pm$ 0.12	1.05 $\pm$ 0.14

<sup>\*</sup>: compared with indexes of control group,  $P<0.05$ ; <sup>#</sup>: compared with indexes of HZ group,  $P<0.05$ .

### 3.2 Cytokine content in serum

Analysis of cytokines NSE (pg/mL), S100B (pg/mL), IFN- $\gamma$  (ng/mL), TNF- $\alpha$  (ng/mL) and IL-17 (ng/mL) contents in serum among the three groups of subjects was as follows: NSE, S100B, IFN- $\gamma$ , TNF- $\alpha$  and IL-17 levels in serum of PHN group and HZ group were significantly higher than those of control group; NSE, S100B, IFN- $\gamma$ , TNF- $\alpha$  and IL-17 levels in serum of PHN group were significantly higher than those of HZ group. Differences in pair-wise comparison of NSE, S100B, IFN- $\gamma$ , TNF- $\alpha$  and IL-17 levels in serum were statistically significant among the three groups of subjects ( $P<0.05$ ). Pearson correlation analysis showed that p38MAPK, ERK1/2 and JNK expression in peripheral blood of PHN patients were positively correlated with NSE, S100B, IFN- $\gamma$ , TNF- $\alpha$  and IL-17 levels in serum.

**Table 3.**

Serum pain mediator contents in three groups of subjects.

Groups	n	5-HT	CGRP	SP	$\beta$ -EP
PHN group	39	394.5 $\pm$ 56.7 <sup>*#</sup>	528.9 $\pm$ 72.4 <sup>*#</sup>	189.5 $\pm$ 20.3 <sup>*#</sup>	30.5 $\pm$ 4.6 <sup>*#</sup>
HZ group	45	226.8 $\pm$ 31.5 <sup>*</sup>	334.5 $\pm$ 42.6 <sup>*</sup>	103.5 $\pm$ 12.7 <sup>*</sup>	48.7 $\pm$ 7.2 <sup>*</sup>
Control group	50	132.5 $\pm$ 17.8	170.5 $\pm$ 20.3	68.9 $\pm$ 8.3	60.4 $\pm$ 7.8

\*: compared with indexes of control group,  $P < 0.05$ ; #: compared with indexes of HZ group,  $P < 0.05$ .

### 3.3 Pain mediator contents in serum

Analysis of pain mediators 5-HT (ng/mL), CGRP (ng/mL), SP (pg/mL) and  $\beta$ -EP (pg/mL) contents in serum among the three groups of subjects was as follows: 5-HT, CGRP and SP levels in serum of PHN group and HZ group were significantly higher than those of control group while  $\beta$ -EP levels were significantly lower than that of control group; 5-HT, CGRP and SP levels in serum of PHN group were significantly higher than those of HZ group while  $\beta$ -EP level was significantly lower than that of HZ group. Differences in pairwise comparison of 5-HT, CGRP, SP and  $\beta$ -EP levels in serum were statistically significant among the three groups of subjects ( $P < 0.05$ ). Pearson correlation analysis showed that p38MAPK, ERK1/2 and JNK expression in peripheral blood of PHN patients were positively correlated with CGRP, 5-HT and SP levels in serum, and negatively correlated with  $\beta$ -EP level in serum.

## 4. Discussion

The pathogenesis of post-herpetic neuralgia (PHN) is not clear, and there is no effective treatment for it. Persistent pain can cause serious effect on the patients' daily life. Inflammatory response and immune response abnormality in local tissues after herpes virus infection are important factors causing nerve injury and neuropathic pain[4,5]. MAPKs signaling pathway is an important pathway regulating inflammatory response and immune response in the body. p38 MAPK, ERK1/2 and JNK pathways are different subtypes of MAPKs pathway. Under the action of chemical factor stimuli, pathogen stimuli, physical factor stimuli and so on, p38 MAPK, ERK1/2 and JNK pathways are activated and influence the expression of a variety of downstream cytokines to participate in the regulation of inflammatory response and immune response[6-8]. In the study, analysis of MAPK signal molecules p38 MAPK, ERK1/2 and JNK expression in peripheral blood after herpes zoster virus infection showed that p38 MAPK, ERK1/2 and JNK expression in peripheral blood of PHN group and HZ group significantly increased and the changes of above MAPK signal molecule expression in

peripheral blood of PHN group were more significant than those of HZ group. This indicates that the activation of p38 MAPK, ERK1/2 and JNK signaling pathways is related to the occurrence of PHN, and activating inflammatory response may be the molecular pathway of MAPK signaling pathway to participate in the development of PHN.

Neuronal inflammation is an important pathological feature of PHN, and p38MAPK, ERK1/2, JNK and so on are the important signaling pathways regulating inflammatory response in the body. The secretion of various nerve cytokines and inflammatory cytokines changes in the process of inflammation of the neuron. NSE and S100B are the important functional molecules in nerve cells, the former is mainly located in neurons and participates in the process of glycolysis energy in the cells, and the latter is mainly located in glial cells and participates in the formation of the skeleton structure; the damage to neurons and glial cells can lead to increased release of NSE and S100. IFN- $\gamma$ , TNF- $\alpha$  and IL-17 are the inflammatory cytokines secreted by the CD4+T cell subgroup, they participate in the regulation of inflammatory response and immune response in local nerve tissue, IFN- $\gamma$  and TNF- $\alpha$  are synthesized and secreted by Th1 cells, and IL-17 is synthesized and secreted by Th17 cells[9]. Th1 and Th17 cells mainly mediate the cellular immune response. Excessive secretion of IFN- $\gamma$ , TNF- $\alpha$  and IL-17 can directly cause damage to local tissues[10,11]. In the study, analysis of the changes of nerve cytokines and inflammatory cytokines after herpes zoster virus infection showed that serum NSE, S100B, IFN- $\gamma$ , TNF- $\alpha$  and IL-17 secretion significantly increased in PHN group and HZ group, and the changes of above cytokine in PHN group were more significant than those in HZ group. This indicates that the abnormal secretion of nerve cytokines and inflammatory cytokines is closely related to the occurrence of PHN. Further analysis of the correlation between MAPK signaling pathway and cytokines showed that p38MAPK, ERK1/2 and JNK expression in peripheral blood of PHN patients were positively correlated with NSE, S100B, IFN- $\gamma$ , TNF- $\alpha$  and IL-17 levels in serum. Therefore, the abnormally activated MAPK signaling pathway in PHN patients can increase the secretion of nerve cytokines and inflammatory cytokines.

The activation of a variety of MAPK signal pathways and the excessive secretion of inflammatory cytokines and nerve cytokines

in PHN patients can cause pain, and the secretion of CGRP, 5-HT, SP,  $\beta$ -EP and various other pain mediators is also abnormal in the process[12]. CGRP is a neuropeptide containing 37 amino acids, and the CGRP- $\beta$  is mainly distributed in the neurons of the spinal cord and peripheral nerve tissue, and it has significant algogenic effect[13,14]; 5-HT is a neurotransmitter with both analgesic and algogenic activity, which has significant algogenic activity in peripheral tissue and can aggravate the pain degree in the course of herpes zoster; SP is a kind of tachykinin that conducts nerve impulses during the production of pain, and the temperature stimulation and biochemical stimulation will increase the expression of SP and increase the conduction of the pain signal[15];  $\beta$ -EP is an important endogenous opioid neuropeptide, which can block the conduction of pain signal impulse and the generation of pain transmitter SP, and has powerful analgesic effect. In the study, analysis of the changes of pain mediators after herpes zoster virus infection showed that serum algogenic mediators CGRP, 5-HT and SP secretion significantly increased while analgesic mediator  $\beta$ -EP secretion significantly decreased in PHN group and HZ group, and the changes of above pain mediators in PHN group were more significant than those in HZ group. This indicates that the abnormal secretion of pain mediators is closely related to the occurrence of PHN. Further analysis of the correlation between MAPK signaling pathways and pain mediators showed that p38MAPK, ERK1/2 and JNK expression in peripheral blood were positively correlated with CGRP, 5-HT and SP levels in serum, and negatively correlated with  $\beta$ -EP level in serum. Therefore, the abnormally activated MAPK signaling pathway in PHN patients can increase the secretion of algogenic mediators and decrease the secretion of analgesic mediators.

The excessive activation of MAPKs signaling pathway is closely related to the occurrence of PHN; the high expression of peripheral blood signal molecules p38MAPK, ERK1/2 and JNK can increase the secretion of cytokines and algogenic mediators and decrease the secretion of analgesic mediators.

## References

- [1] Wollina U, Machetanz J. Herpes zoster and postherpetic neuralgia. *Hautarzt* 2016; **67**(8): 653-665.
- [2] Mallick-Searle T, Snodgrass B, Brant JM. Postherpetic neuralgia: epidemiology, pathophysiology, and pain management pharmacology. *J Multidiscip Healthc* 2016; **21**(9): 447-454.
- [3] Silva JR, Lopes AH, Talbot J, Cecilio NT, Rossato MF, Silva RL, et al. Neuroimmune-glia interactions in the sensory ganglia account for the development of acute herpetic neuralgia. *J Neurosci* 2017; **37**(27): 6408-6422.
- [4] Friesen KJ, Falk J, Alessi-Severini S, Chateau D, Bugden S. Price of pain: population-based cohort burden of disease analysis of medication cost of herpes zoster and postherpetic neuralgia. *J Pain Res* 2016; **3**(9): 543-550.
- [5] Wu CH, Chai CY, Tung YC, Lu YY, Su YF, Tsai TH, et al. Herpes zoster as a risk factor for osteoporosis: A 15-year nationwide population-based study. *Medicine (Baltimore)* 2016; **95**(25): e3943.
- [6] Wang K, Wang F, Bao JP, Xie ZY, Chen L, Zhou BY, et al. Tumor necrosis factor  $\alpha$  modulates sodium-activated potassium channel SLICK in rat dorsal horn neurons via p38 MAPK activation pathway. *J Pain Res* 2017; **25**(10): 1265-1271.
- [7] Chen NF, Chen WF, Sung CS, Lu CH, Chen CL, Hung HC, et al. Contributions of p38 and ERK to the antinociceptive effects of TGF- $\beta$  1 in chronic constriction injury-induced neuropathic rats. *J Headache Pain* 2016; **17**(1): 72.
- [8] Worsley MA, Allen CE, Billinton A, King AE, Boissonade FM. Chronic tooth pulp inflammation induces persistent expression of phosphorylated ERK (pERK) and phosphorylated p38 (pp38) in trigeminal subnucleus caudalis. *Neuroscience* 2014; **6**(269): 318-330.
- [9] Jing L, Laing KJ, Dong L, Russell RM, Barlow RS, Haas JG, et al. Extensive CD4 and CD8 T cell cross-reactivity between alphaherpesviruses. *J Immunol* 2016; **196**(5): 2205-2218.
- [10] Chlibek R, Pauksens K, Rombo L, van Rijckevorsel G, Richardus JH, Plassmann G, et al. Long-term immunogenicity and safety of an investigational herpes zoster subunit vaccine in older adults. *Vaccine* 2016; **34**(6): 863-868.
- [11] Lees JG, Duffy SS, Perera CJ, Moalem-Taylor G. Depletion of Foxp3+ regulatory T cells increases severity of mechanical allodynia and significantly alters systemic cytokine levels following peripheral nerve injury. *Cytokine* 2015; **71**(2): 207-214.
- [12] Guedon JM, Yee MB, Zhang M, Harvey SA, Goins WF, Kinchington PR. Neuronal changes induced by Varicella Zoster Virus in a rat model of postherpetic neuralgia. *Virology* 2015; **482**: 167-180.
- [13] Walsh DA, Mapp PI, Kelly S. Calcitonin gene-related peptide in the joint: contributions to pain and inflammation. *Br J Clin Pharmacol* 2015; **80**(5): 965-978.
- [14] Nieto FR, Clark AK, Grist J, Chapman V, Malcangio M. Calcitonin gene-related peptide-expressing sensory neurons and spinal microglial reactivity contribute to pain states in collagen-induced arthritis. *Arthritis Rheumatol* 2015; **67**(6): 1668-1677.
- [15] Nicodemi S, Corelli S, Sacchi M, Ricciardi E, Costantino A, Di Legge P, et al. Recurrent incisional hernia, enterocutaneous fistula and loss of the substance of the abdominal wall: plastic with organic prosthesis, skin graft and VAC therapy. *Annali Italiani Di Chirurgia* 2015; **86**(2): 172-176.