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Analysis of the pain and neuroinflammation after rats with neuropathic pain receive immunoglobulin intervention

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ABSTRACT

Objective: To study the effect of immunoglobulin intervention on the pain and neuroinflammation in rats with neuropathic pain. **Methods:** Wistar rats were selected as experimental animals and randomly divided into control group (Sham group), neuropathic pain model group (NP) and immunoglobulin intervention group (IG), NP group and IG group were made into neuropathic pain rat models, and the IG group received 0.4 g/kg immunoglobulin intervention. 21 d after intervention, the levels of pain mediators, pro-inflammatory factors and anti-inflammatory factors in serum and spinal cord were detected. **Results:** Serum and spinal cord SP, 5-HT, GABA, IFN-γ, TNF-α and IL-1β levels of NP group were significantly higher than those of Sham group (P<0.05) while serum and spinal cord IL-4 and IL-10 levels were significantly lower than those of Sham group (P<0.05); serum and spinal cord SP, 5-HT, GABA, IFN-γ, TNF-α and IL-1β levels of IG group were significantly lower than those of NP group (P<0.05) while serum and spinal cord IL-4 and IL-10 levels were significantly higher than those of NP group (P<0.05). **Conclusion:** Immunoglobulin can relieve the pain and suppress the neuroinflammation in rats with neuropathic pain.

1. Introduction

Neuropathic pain (NP) is a type of pain characterized by hyperalgesia and spontaneous pain, and cancer, diabetes, autoimmune disease and nerve compression are the common causes of NP[1]. The peripheral and central sensitization caused by neural immune system disorders and the abnormal secretion of inflammatory mediators is a key link that causes neuropathic pain, and this process is also called "neurogenic inflammation"[2,3]. Clinical researches have shown that intravenous immunoglobulin has good analgesic effect on the neuropathic pain caused by autoimmune disease and diabetes[4,5]. However, the concrete mechanism for immunoglobulin to intervene with and relieve neuropathic pain is not yet clear at present, and it remains to be further confirmed whether the immunoglobulin can inhibit the neurogenic inflammation during neuropathic pain to exert analgesic effect. In the following study, neuropathic pain model rats were selected as research objects, and the effect of immunoglobulin intervention on the pain and neuroinflammation in rats with neuropathic pain was analyzed.

2. Materials and methods

2.1. Experimental materials

36 adult and male Wistar rats were bought from the laboratory animal center of Second Military Medical University, the animal license number: SCXK (Shanghai) 2012-0002, and the body weight was 250–300 g; experimental equipment and routine reagents were provided by laboratory animal center, enzyme-linked immunosorbent assay kits were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd, and the protein lysis buffer and BCA protein quantification kits were bought from Shanghai Beyotime Company.

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2.2. Experimental methods

2.2.1. Animal grouping and model making methods

Wistar rats were randomly divided into control group (Sham group), neuropathic pain model group (NP group) and immunoglobulin intervention group (IG group), 12 in each group. NP group and IG group were made into neuropathic pain models as follows: after intraperitoneal injection of sodium pentobarbital for anesthesia, the skin of back was cut open to separate muscle and expose the transverse process of the fifth lumbar, rongeur was used to open the transverse process of lumbar and expose spinal nerve and ligature it with the thread, and then the incision was sutured. Sham group received spinal nerve exposure in the same way as that of NP group and IG group, no ligation was done, and the incision was sutured directly.

2.2.2. Drug intervention methods

Drug intervention was done 15 min after model making, IG group received immunoglobulin injection through caudal vein with the dose of 0.4 g/kg, and the Sham group and NP group received the same dose of saline injection through caudal vein, once a day, for 21 d in a row.

2.2.3. Serum sample collection and index detection methods

21 d after drug intervention, rats were put to death by decapitation, blood samples were collected immediately, let stand at room temperature for about 30 min for natural coagulation, and then centrifuged for 10 min at a speed of 1 000 r/min to separate serum, and enzyme-linked immunosorbent assay kits were used to determine SP, 5-HT, GABA, IFN-γ, TNF-α, IL-1β, IL-4 and IL-10 levels.

2.2.4. Spinal cord sample collection and index detection methods

21 d after drug intervention, the rats were executed, the spinal cord tissue corresponding to the fifth lumbar vertebra was separated, washed with saline, added in 0.3 mL protein lysis buffer, fully grinded in high-flux tissue blender and centrifuged to separate supernatant, enzyme-linked immunosorbent assay kits were used to detect SP, 5-HT, GABA, IFN-γ, TNF-α, IL-1β, IL-4 and IL-10 levels, BCA kits were used to detect total protein content, and SP, 5-HT, GABA, IFN-γ, TNF-α, IL-1β, IL-4 and IL-10 contents per mg total protein were calculated.

2.3. Statistical analysis

SPSS21.0 software was used to input the detected data of serum samples and spinal cord samples, differences in above data among three groups was by variance analysis, and $P<0.05$ indicated statistical significance in differences.

3. Results

3.1. Serum and spinal cord pain mediator levels

Analysis of serum pain mediators SP, 5-HT and GABA levels as well as spinal cord SP, 5-HT and GABA levels among three groups of rats was as follows: serum and spinal cord SP, 5-HT and GABA levels of NP group were significantly higher than those of Sham group; serum and spinal cord SP, 5-HT and GABA levels of IG group were significantly lower than those of NP group. Differences in pair-wise comparison of serum and spinal cord SP, 5-HT, GABA levels were statistically significant among three groups of rats ($P<0.05$) (Table 1).

3.2. Serum and spinal cord inflammatory factor levels

Analysis of the serum pro-inflammatory factors IFN-γ, TNF-α and IL-1β, serum anti-inflammatory factors IL-4 and IL-10 levels as well as spinal cord IFN-γ, TNF-α, IL-1β, IL-4 and IL-10 levels among three groups of rats was as follows: serum and spinal cord IFN-γ, TNF-α and IL-1β levels of NP group were significantly higher than those of Sham group while IL-4 and IL-10 levels were significantly lower than those of Sham group; serum and spinal cord IFN-γ, TNF-α and IL-1β levels of IG group were significantly lower than those of NP group while IL-4 and IL-10 levels were significantly higher than those of NP group. Differences in pair-wise comparison of serum and spinal cord IFN-γ, TNF-α, IL-1β, IL-4 and IL-10 levels were statistically significant among three groups of rats ($P<0.05$) (Table 2).

Table 1

Comparison of pain mediator levels among three groups of rats (n=12, ng/mL,$\bar{x}\pm s$)

Groups	Serum levels			Spinal cord levels		
	SP	5-HT	GABA	SP	5-HT	GABA
Sham group	1.85±0.22	1.02±0.15	2.25±0.39	0.92±0.11	0.72±0.09	1.94±0.29
NP group	5.48±0.89[a]	4.27±0.62[a]	8.49±1.15[a]	2.47±0.42[a]	2.18±0.39[a]	4.49±0.65[a]
IG group	2.77±0.39[b]	1.89±0.26[b]	3.77±0.52[b]	1.65±0.25[b]	1.34±0.21[b]	2.71±0.38[b]

[a]: comparison between NP group and Sham group, $P<0.05$; [b]: comparison between IG group and NP group, $P<0.05$.

Table 2

Comparison of serum inflammatory factor levels among three groups of rats (n=12, ng/mL, $\bar{x}\pm s$).

Groups	Serum levels					Spinal cord levels				
	IFN -γ	TNF-α	IL-1β	IL-4	IL-10	IFN -γ	TNF-α	IL-1β	IL-4	IL-10
Sham group	3.82±0.52	6.37±0.89	1.68±0.22	3.18±0.52	2.47±0.35	0.95±0.11	2.31±0.42	0.72±0.10	2.94±0.41	1.25±0.19
NP group	11.29±1.85[a]	18.22±3.14[a]	6.68±0.93[a]	1.02±0.17[a]	0.83±0.11[a]	3.26±0.51[a]	9.48±1.16[a]	2.04±0.38[a]	0.98±0.12[a]	0.42±0.07[a]
IG group	5.76±0.74[b]	9.38±1.16[b]	3.02±0.47[b]	2.28±0.35[b]	1.89±0.27[b]	1.44±0.19[b]	3.76±0.52[b]	1.13±0.16[b]	1.54±0.24[b]	0.98±0.13[b]

[a]: comparison between NP group and Sham group, P<0.05; [b]: comparison between IG group and NP group, P<0.05.

4. Discussion

Neural immune dysfunction and neurogenic inflammation is one of the mechanisms of neuropathic pain. Mechanical pressure, hyperglycemia, autoantibody and other nociceptive stimuli can act on nerve tissue and activate the inflammation, which leads to the abnormal secretion of the inflammatory mediators and also causes the abnormal secretion of pain mediators[6,7]. Immunoglobulin is an important active material to adjust the immune function and nerve inflammation, the immunoglobulin secreted by neurons and glial cells have neuroprotective effect, and in pathological conditions, it can be combined with FcγRI on the cell membrane to reduce the immune disorders and the sensitization of inflammatory activation on neural function. Clinical studies have shown that intravenous immunoglobulin has good analgesic effect on the neuropathic pain caused by autoimmune disease and diabetes[4,5]. The study of domestic Wang *et al*[8] confirms that immunoglobulin can relieve the pain in rats with neuropathic pain, which is characterized by the increased paw withdrawal mechanical threshold and the extended paw withdrawal thermal latency. This suggests that the immunoglobulin plays an analgesic role in the pathological process of neuropathic pain.

The occurrence of pain perception is associated with the abnormal synthesis and secretion of pain mediators, and during the generation of neuropathic pain, the activation of neurogenic inflammation can promote the abnormal secretion of pain mediators. In the study, in order to define the immunoglobulin effect on neuropathic pain, the pain mediator levels in serum and spinal cord were analyzed. Substance P is a kind of tachykinin, and its combination with membrane receptor NK-1 can increase the discharge of neurons and generate pain perception[9,10]; 5-HT combination with the receptors on the spinal dorsal horn neurons can start the inward current mediated by sodium and calcium, causing the increased pain sensitivity[11]; GABA is an inhibitory neurotransmitter, and it can act on the synaptic structure of neurons between spinal cord to weaken the body's inhibitory effect on noxious stimulation and cause pain perception[12]. Analysis of above pain mediator levels in the study showed that serum and spinal cord SP, 5-HT and GABA levels of NP group were significantly higher than those of Sham group (P<0.05); serum and spinal cord SP, 5-HT and GABA levels of IG group were significantly lower than those of NP group (P<0.05). This means that abnormally secreted pain mediators SP, 5-HT and GABA are involved in the generation of neuropathic pain, and intravenous immunoglobulin can inhibit the secretion of pain mediators and relieve pain.

The activation of neurogenic inflammation is a key link that causes neuropathic pain, characterized by the secretion disorder of a variety of pro-inflammatory factors and anti-inflammatory factors[6,7,13]. IFN-γ, TNF-α and IL-1β are pro-inflammatory factors closely related to neuropathic pain. IFN-γ is mainly secreted by the Th1 cells infiltrating in nervous tissue, and it can increase the expression of iNOS and P2X4 receptors, and cause pain perception; TNF-α and IL-1β are mainly from macrophages and mast cells, the former acts on the membrane receptors TNFR1 and TNFR2 in dorsal root ganglion and spinal cord dorsal horn to play its part[14], and the latter plays its part after maturation by MMPs cutting[10,15]. The two can cause inflammation cascade amplification together, reduce the neuronal excitation threshold and increase pain sensitivity. IL-4 and IL-10 are two main anti-inflammatory factors in the body, and they can be combined with the corresponding membrane receptors to activate the signaling pathways in JAKs/STATs cells, and thereby inhibit the expression and secretion of a variety of inflammatory mediators[16,17]. In the study, analysis of the levels of above pro-inflammatory factors and anti-inflammatory factors in serum and spinal cord proved that serum and spinal cord IFN-γ, TNF-α and IL-1β levels of IG group were significantly lower than those of NP group (P<0.05) while serum and spinal cord IL-4 and IL-10 levels were significantly higher than those of NP group (P<0.05). This means that the immunoglobulin can regulate the inflammatory reaction in the course of neuropathic pain, which is specifically characterized by inhibiting the secretion of pro-inflammatory factors, increase the secretion of anti-inflammatory factors, and thereby inhibiting the activation of inflammatory response.

In the study, neuropathic pain model rats were selected as the research objects to analyze the influence of immunoglobulin on neuropathic pain, and in the occurrence of neuropathic pain, the secretion of pain mediators increases and the inflammatory response is abnormally activated; intravenous immunoglobulin can inhibit the secretion of pain mediators as well as the activation of inflammation, reduce the secretion of pro-inflammatory factors

and increase the secretion of anti-inflammatory factors. Although the effect of immunoglobulin on relieving neuropathic pain is very exact, the specific mechanism for the immunoglobulin to inhibit the inflammation during neuropathic pain remains to be further explored.

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