Effect of lycopene on the expression of pain-related molecules in spinal cord of model rats with neuropathic pain

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ABSTRACT

Objective: To analyze the effect of lycopene on the expression of pain-related molecules in spinal cord of model rats with neuropathic pain. Methods: A total of 30 healthy female SD rats were collected to establish neuropathic pain models according to the literatures, including 10 in sham operation group, 10 in model control group and 10 in model treatment group. Rats were executed to obtain L2-L6 segment of spinal cord, and then serum levels of pain-related indicators as well as gene and protein expression in it were detected. Results: Serum IL-17, HMGB-1, Aβ, Tau and C3 levels of sham operation group were lower than those of model control group and model treatment group while CGRP level was higher than that of model control group and model treatment group, and serum IL-17, HMGB-1, Aβ, Tau and C3 levels of model treatment group were lower than those of model control group while CGRP level was higher than that of model control group and model treatment group; ERK, CREB, BDNF, NMDA, AMPA and c-fos mRNA expression levels of sham operation group were lower than those of model control group and model treatment group while CGRP level was higher than that of model control group and model treatment group, and ERK, CREB, BDNF, NMDA, AMPA and c-fos mRNA expression levels of model treatment group were lower than those of model control group and model treatment group, and TRPV1, NF-κB, NOS, GFAP, ERK and CREB protein expression levels of sham operation group were lower than those of model control group and model treatment group while Reg expression level was higher than that of model control group and model treatment group, and TRPV1, NF-κB, NOS, GFAP, ERK and CREB protein expression levels of model treatment group were lower than those of model control group while Reg expression level was higher than that of model control group. Conclusion: Lycopene can effectively decrease the expression of pain-promoting genes in model rats with neuropathic pain, and is expected to become new treatment means of neuropathic pain in the future.

1. Introduction

Neuropathic pain (NPP) is one of the most common clinical chronic pain, it seriously influences patients’ quality of life and normal social function, but its etiology is numerous, targeted treatments are few, and it becomes a major clinical treatment problem. Lycopene is a natural pigment in plants, is one of the strongest natural antioxidants, can effectively remove free radicals in the body, and has a positive role in preventing and controlling aging and inhibiting decline of immunity[1,2]. The latest research shows that lycopene also has a certain role in the aspect of treating NPP, but the specific mechanism is unclear. In the research, the effect of lycopene on the expression of pain-related molecules in spinal cord of model rats with neuropathic pain was mainly analyzed, hereby reported as follows.

2. Research materials and methods

2.1 Experimental animals

Animal study was approved by the hospital ethic committee,
the animals used were 30 healthy female SD rats with body mass 180-200 g, and they were all purchased by the university animal center from Shanghai Slac Laboratory Animal Co., Ltd. 12 h of illumination with alternating light and darkness was conducted, with free food and drinking water, etc, ensuring quiet environment, with good ventilation and air filter conditions, and controlling room temperature at 20 °C and humidity about 50%. After environmental adaptation for a week, rats were divided into sham operation group, model control group and model treatment group, 10 in each group.

2.2 Model establishment and medication

Establishment of neuropathic pain rat models: after intraperitoneal injection of 2% pentobarbital anesthesia, rats lay on side and were fixed on the operating table, preserved right thigh skin was disinfected and incised to bluntly dissect muscles and expose 10 mm proximal sciatic nerve, mild ligation rings were made around nerve trunk, ring spacing was about 1 mm to ensure that the epineurium was mildly sunk and avoid nerve damage from tight ligation, and the skin and muscle were sutured step by step. For sham operation group, after right thigh skin incision and blunt muscle separation, nerve ligation was not conducted, and muscle and skin were sutured after disinfection. Model treatment group received intragastric administration of 40 mg/kg lycopene on daily basis for operation for continuous 14 d; model control group while CGRP level was higher than that of model control group and model treatment group were lower than those of model control group and model treatment group while CGRP level was lower than that of model control group while model treatment group were lower than those of model control group while CGRP level was higher than that of model control group (P<0.05), shown in Table 1.

2.3 ELISA detection

Rat spinal cord was homogenized and centrifuged with 4 000 r/min for 10 min, pipette was used to collect supernatant, put it in -20 °C refrigerator and set aside. The levels of interleukin-17 (IL-17), high mobility group box 1 (HMGB-1), β-amyloid (A β), nerve microtubule-associated protein (Tau), complement C, and calcitonin gene-related peptide (CGRP) in it were detected.

2.4 Fluorescence quantitative PCR detection

Rats were decapitated, then L2-L6 segment of spinal cord was rapidly collected, homogenized in ice bath and centrifuged, and ERK, CREB and BDNF mRNA, ionic glutamate receptors (iGluR) NMDA and AMPA mRNA as well as c-fos mRNA expression levels in it were detected.

2.5 Western–blot detection

Transient receptor potential vanilloid-1 (TRPV1), nuclear factor kappa B (NF-κ B), nitric oxide synthase (NOS), regenerating gene (Reg), glial fiber acidic protein (GFAP), extracellular signal-regulated kinase (ERK) and cAMP response element-binding protein (CREB) protein expression levels in spinal cord L2-L6 homogenate were detected.

2.6 Statistical methods

Data obtained in the research was analyzed by SPSS 23.0 software, measurement data was in terms of Mean±SD, comparison between two groups was by t test and P<0.05 was set as the standard of statistical significance in differences.

3. Results

3.1 Serum related indicators

Differences in serum IL-17, HMGB-1, A β , Tau, C, and CGRP levels of three groups were statistically different (P<0.05), serum IL-17, HMGB-1, A β , Tau and C levels of sham operation group were lower than those of model control group and model treatment group while CGRP level was higher than that of model control group and model treatment group, and serum IL-17, HMGB-1, A β , Tau and C levels of model treatment group were lower than those of model control group while CGRP level was higher than that of model control group (P<0.05), shown in Table 1.

3.2 Spinal cord mRNA expressions

Differences in spinal cord ERK, CREB, BDNF, NMDA, AMPA and c-fos mRNA expression levels of three groups were statistically different (P<0.05), ERK, CREB, BDNF, NMDA, AMPA and c-fos mRNA expression levels of sham operation group were lower than those of model control group and model treatment group, and spinal cord ERK, CREB, BDNF, NMDA, AMPA and c-fos mRNA expression levels of model treatment group were lower than those of model control group (P<0.05), shown in Table 2.

3.3 Spinal cord pain–related protein expressions

Differences in spinal cord TRPV1, NF-κ B, NOS, Reg, GFAP, ERK and CREB protein expression levels of three groups were statistically different (P<0.05), TRPV1, NF-κ B, NOS, GFAP, ERK and CREB protein expression levels of sham operation group were lower than those of model control group and model treatment group while Reg expression level was higher than that of model control group and model treatment group (P<0.05), and TRPV1, NF-κ B, NOS, GFAP, ERK and CREB protein expression levels of model treatment group were lower than those of model control group while Reg expression level was higher than that of model control group (P<0.05), shown in Table 3.

Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-17 (ng/L)</th>
<th>HMGB-1 (pg/L)</th>
<th>A β (μg/L)</th>
<th>Tau (μg/L)</th>
<th>C (g/L)</th>
<th>CGRP (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>23.17±2.04</td>
<td>1.36±0.12</td>
<td>153.28±11.23</td>
<td>21.37±2.04</td>
<td>0.21±0.03</td>
<td>533.82±50.76</td>
</tr>
<tr>
<td>Model control</td>
<td>112.47±10.94</td>
<td>14.09±1.35</td>
<td>375.09±34.28</td>
<td>112.48±10.76</td>
<td>0.63±0.05</td>
<td>163.27±11.19</td>
</tr>
<tr>
<td>Model treatment</td>
<td>78.39±6.95</td>
<td>8.11±0.79</td>
<td>223.84±19.73</td>
<td>58.39±5.42</td>
<td>0.42±0.04</td>
<td>374.87±29.68</td>
</tr>
<tr>
<td>F</td>
<td>7.495</td>
<td>6.382</td>
<td>11.204</td>
<td>9.384</td>
<td>5.182</td>
<td>9.394</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Establishment of model control group and model treatment group, 10 in each group.
secretion of TNF-α and IFN-γ, the proliferation of neutrophils and monocytes, and increase the response, histocyte damage and multiple links. IL-17 can promote factor involved in the cascade amplification of inflammatory Interleukin-17 (IL-17) is a newly discovered pro-inflammatory effective.

In order to make clear whether lycopene treatment of NNP was and protein expression were compared under different intervention model rats were established in this study, and pain-related gene neuropathic pain and its mechanism of action, neuropathic pain treatment, and it plays a positive role in easing patients' perception of pain.

Neuropathic pain (NPP) is associated with a variety of peripheral nerve disorders, and it is the pain caused by primary lesion or dysfunction of nervous system. The occurrence of NPP is related to inflammatory mediators, central sensitization, central disinhibition, NMDA receptor and spinal opioid system down-regulation, and it is a severe disease causing persistent pain and lower quality of life in many patients[3]. Current treatment of neuropathic pain needs effective means, lycopene belongs to the carotenoids, and it can play oxygen free radicals, increasing immunity, inducing intercellular communication and so on. Currently there are studies that have attempted to apply the lycopene in the treatment of patients with neuropathic pain and have acquired certain therapeutic effect, so it is believed that lycopene can be used as a new way of neuropathic pain treatment, and it plays a positive role in easing patients' perception of pain[4,5]. In order to define the concrete effect of lycopene on neuropathic pain and its mechanism of action, neuropathic pain model rats were established in this study, and pain-related gene and protein expression were compared under different intervention in order to make clear whether lycopene treatment of NNP was effective.

Interleukin-17 (IL-17) is a newly discovered pro-inflammatory factor involved in the cascade amplification of inflammatory response, histocyte damage and multiple links. IL-17 can promote the proliferation of neutrophils and monocytes, and increase the secretion of TNF- and IFN-γ, etc. High mobility group box 1 (HMGB-1) is an inflammatory mediator that plays a part in the late inflammation, and it can activate the release of a variety of inflammatory cells and inflammatory cytokines[6]. β-amyloid (Aβ) is from β-amyloid precursor protein hydrolysis, and has very strong neurotoxicity. Nerve microtubule-associated protein (Tau) is the cytoskeleton component of nerve cells, and participates in a variety of cellular functions. Both Aβ and Tau are the nervous system proteins closely associated with cognitive impairment, and can sensitively reflect nervous system damage. Aβ has direct cell damage effect, which induces calcium overload, inflammation reaction activation and so on to damage cellular homeostasis. Tau is released into the blood circulation in cases of neuron injury, and is directly proportional to the degree of nerve injury and pain in patients[7,8]. Complement system is an important part of the body's defense system, C3 content is the most abundant in the body fluid, C3 cracking fragment and binding protein play an important role in immune defense and immune regulation, C3 is regarded as an important index of complement activation, and after nerve injury, the expression of large amounts of C3 can be detected in degenerated myelin and axon. Calcitonin gene-related peptide (CGRP) is a neuropeptide widespread in central and peripheral nervous system, and it exerts analgesic effect at supraspinal level. A study shows that CGRP expression decreases in patients with central pain, suggesting that CGRP levels are negatively correlated with the degree of pain[9]. Above research results showed that there were high expression of IL-17, HMGB-1, Aβ, Tau and C3, as well as low expression of CGRP in NPP model rats, and after intragastric administration of lycopene, IL-17, HMGB-1, Aβ, Tau and C3 expression decreased while CGRP expression increased, approaching the levels of sham operation group, and it indicated that lycopene was of positive significance in optimizing the levels of pain-related factors.

ERK, CREB and BDNF are widely distributed in the brain and can adjust nervous system development and maturation, and BDNF combination with target tissue cell membrane receptor can promote TrkB dimer formation, sequentially activate a variety of proteins and enzymes, start phosphatase C and other gene transcription as well as protect and promote the regeneration of neurons[10]. It has been found that BDNF expression decreases in neuropathic pain model rats, which is one of the important factors causing the occurrence and aggravation of pain. After abnormally activated, ionic glutamate receptors (Glur) NMDA and AMPA form "winding up" phenomenon through intercellular cascade mediated by calcium ion, lead to the occurrence and maintenance of the central sensitization of the spinal dorsal horn neurons, and are essential for neuropathic pain. c-fos proto-oncogene-expressed FOS protein is considered as the sign of excitabile activity of neurons, and studies show that analgesic drug application can lead to the reduction of spinal cord c-fos expression, indicating that c-fos is one of the precipitating factors of pain occurrence[11,12]. In above research, ERK, CREB, BDNF, NMDA, AMPA and c-fos mRNA expression levels increased in spinal cord of NPP model rats, it might be the important factors of pain occurrence, the expression levels of above genes significantly decreased in model treatment group after lycopene treatment.

Table 2
Comparison of spinal cord mRNA expression levels among three groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>ERK</th>
<th>CREB</th>
<th>BDNF</th>
<th>NMDA</th>
<th>AMPA</th>
<th>c-fos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>100.00±8.96</td>
<td>100.00±8.96</td>
<td>100.00±8.96</td>
<td>100.00±8.96</td>
<td>100.00±8.96</td>
<td>100.00±8.96</td>
</tr>
<tr>
<td>Model control</td>
<td>193.27±16.83</td>
<td>212.83±19.55</td>
<td>173.26±16.03</td>
<td>186.32±15.17</td>
<td>188.66±17.52</td>
<td>203.81±19.73</td>
</tr>
<tr>
<td>Model treatment</td>
<td>132.38±11.17</td>
<td>153.29±13.74</td>
<td>121.85±10.95</td>
<td>134.27±12.88</td>
<td>136.95±13.11</td>
<td>163.29±14.27</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 3
Comparison of spinal cord pain-related protein expression among three groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>TRPV 1</th>
<th>NF-κB</th>
<th>NOS</th>
<th>Reg</th>
<th>GFAP</th>
<th>ERK</th>
<th>CREB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>100.00±8.39</td>
<td>100.00±9.12</td>
<td>100.00±7.28</td>
<td>100.00±9.34</td>
<td>100.00±7.49</td>
<td>100.00±10.34</td>
<td>100.00±9.64</td>
</tr>
<tr>
<td>Model control</td>
<td>183.92±16.48</td>
<td>163.28±15.28</td>
<td>159.63±14.39</td>
<td>58.39±4.77</td>
<td>204.82±18.39</td>
<td>173.26±15.88</td>
<td>166.35±15.83</td>
</tr>
<tr>
<td>Model treatment</td>
<td>134.28±11.04</td>
<td>123.15±10.48</td>
<td>129.47±11.28</td>
<td>82.16±7.93</td>
<td>153.28±13.06</td>
<td>143.99±13.29</td>
<td>143.27±11.24</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

4. Discussion

Neuropathic pain (NPP) is associated with a variety of peripheral nerve disorders, and it is the pain caused by primary lesion or dysfunction of nervous system. The occurrence of NPP is related to inflammatory mediators, central sensitization, central disinhibition, NMDA receptor and spinal opioid system down-regulation, and it is a severe disease causing persistent pain and lower quality of life in many patients[3]. Current treatment of neuropathic pain needs effective means, lycopene belongs to the carotenoids, and it can play...
it directly indicated the huge significance of lycopene in NPP treatment, and it could directly exert the pain-inhibiting effect.

Transient receptor potential vanilloid-1 (TRPV1) is a member of transient receptor potential family and is widely distributed in nociceptive neurons, and activated TRPV1 can regulate calcium influx and trigger nerve endings release of excitatory amino acids, resulting in pain formation in cerebral cortex\[13\]. Nuclear factor-kappa B (NF-κB), as a transcription factor, plays an important role in inflammation, immunity, cell proliferation differentiation and other life activities, and after activated by a variety of stimuli, NF-κB adjusts the expression of many target genes and acute phase proteases. A study shows that intrathecal injection of NF-κB can significantly enhance the mechanical allodynia in rats with sciatic nerve ligation, and NF-κB activation is necessary to produce allodynia\[14\]. Nitric oxide synthase (NOS) has the functions of regulating immunity, adjusting blood pressure, inhibiting platelet aggregation and so on, and as an important intracellular and cell-cell messenger molecule, NOS also has regulating effect on neuropathic pain. NOS is the rate-limiting enzyme of NO that participates in the transmission of nociceptive information at spinal cord level as well as the maintenance of pain modulation and hyperalgesia. Regenerating gene (Reg) has the function similar to that of acute reactive protein, antiapoptotic factor and so on, Reg is expressed in motor nerve and dorsal root ganglia, and Reg expression can rapidly increase in the state of sciatic nerve injury so as to exert neurotrophic factor function and protect the neurons. Many studies have confirmed that astrocytes are activated when neuropathic pain is produced, the expression level of glial fiber acidic protein (GFAP), a specific marker, increases, causing nerve inflammation and immune response, leading to nerve dysfunction, hyperpathia and paralgesia. Extracellular signal-regulated kinase (ERK) is a member of mitogen-activated protein kinase that can mediate the transduction of many intracellular signals, and is related to nociceptive stimulus transmission and nerve sensitization\[15\]. cAMP response element-binding protein (CREB) activation is involved in central and peripheral sensitization after nerve injury. A large number of calcium ions inflows after peripheral nerve injury, and after activating protein kinase, can further activate CREB and increase its expression, and hyperpathia occurs in animal models. Above research results showed that TRPV1, NF-κB, NOS, GFAP, ERK and CREB protein expression levels of NNP model rats increased while Reg protein expression level decreased, and the protein expression of above factors tended to be normal in model treatment group after lycopene treatment, further indicating the reliable value and related action pathways of lycopene in NPP treatment.

To sum up, it is concluded as follows: lycopene can effectively decrease the expression of pain-promoting genes in model rats with neuropathic pain, is expected to become new treatment means of neuropathic pain in the future, and is worth popularization and application in clinical practice in the future.

References