Study on Paeoniflorin Promoting the Recovery of Motor Function of Rat Spinal Cord Injury by Mediating MAPK/ERK and Akt/mTOR Signal Pathway

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Abstract: Spinal cord injury (SCI) is a devastating disease that can cause severe motor, sensory, and autonomic dysfunction. There is currently no effective treatment. Paeonia lactiflora is a traditional Chinese herbal medicine, which has antispasmodic, analgesic, and blood circulation effects. Paeoniflorin (PEF) is a medicinal plant isolated from Paeoniae Radio, and it is widely used in East Asia. A large number of studies have shown that PEF has a powerful neuroprotective effect. However, the potential mechanism of PEF on SCI needs further study. This project uses Basso Beattie Bresnahan (BBB) motor function score and open field test to evaluate neurological function, and uses immunofluorescence method to detect brain-derived neurotrophic factor (BNDF) and neurotrophin-3 (NT). -3) Protein expression, Western blot is used to detect protein expression level, and RT-PCR is used to detect mRNA expression level. Thus, a rat spinal cord injury model was established to observe the effects of AT/mTOR and MAPK/ERK signal pathways activated by PEF on nerve regeneration and functional recovery in rats with spinal cord injury.
***Keywords*:** spinal cord injury, paeoniflorin, recovery

1. Introduction

Spinal cord injury (SCI) is a devastating disease that can cause severe motor, sensory, and autonomic dysfunction. There is currently no effective treatment. The average incidence of spinal cord injury worldwide is estimated to be 1:1000, and the average incidence is 4 to 9 cases per 100,000 population per year. The average incidence of spinal cord injury in developing countries is estimated to be 25.5/million/year, ranging from 2.1 to 130.7/million/year. Spinal cord injury has obvious characteristics and can be divided into two mechanisms: primary mechanical injury and secondary injury. Spinal cord injury is considered to be an incurable disease with uneven recovery and uncertain prognosis, but many methods have opened up new directions for the treatment of spinal cord injury, including surgical decompression, neuroprotective agents, and so on.

Paeonia lactiflora is a traditional Chinese herbal medicine, belonging to the Ranunculaceae family. It has antispasmodic, analgesic, and blood-stimulating effects. Paeoniflorin (PEF) is the main medicinal active ingredient in peony. It is a bicyclic monoterpene glycoside compound. It is a medicinal plant isolated from peony (Paeoniae Radio) and has a wide range of applications in East Asia. A large number of studies have shown that PEF has a powerful neuroprotective effect and can improve central nervous system diseases, such as cognitive impairment, depression and Parkinson's.

Studies have shown that the neuroprotective effect of PEF may be related to the anti-inflammatory and anti-apoptotic effects mediated by TLR4/NF-κB. However, the potential mechanism of PEF on SCI needs further study. This project aims to establish a rat spinal cord injury model to observe the effects of AT/mTOR and MAPK/ERK signal pathways activated by PEF on nerve regeneration and functional recovery in rats with spinal cord injury.

Paeoniflorin is the main active ingredient of the commonly used traditional Chinese medicine Paeonia lactiflora, which belongs to the bicyclic monoterpenoid glycoside compound. In recent years, scholars at home and abroad have carried out more in-depth research on the pharmacological effects of paeoniflorin. They found that paeoniflorin has many functions such as anti-inflammatory, immune regulation and neuroprotection. Its role and potential have been valued by scientific researchers, especially the neuroprotective effect of paeoniflorin is one of the focuses of research in recent years.

In anti-Alzheimer's disease studies, it was found that paeoniflorin can significantly improve the cognitive function of AD transgenic mice, increase the Bcl-2/Bax ratio and the expression level of p-Akt, and down-regulate the expression of

p-p38MAPK to reduce inflammation Reaction and caspase-3 activity. Paeoniflorin can also inhibit the neuroinflammation caused by the GSK-3β, NF-κB signaling pathway and the nucleotide binding domain-like receptor protein 3 (NLRP3) inflammasome, and affect the pathological changes of APP/PS1 transgenic mice. To the effect of anti-inflammatory and anti-Aβ deposition. The activation of microglia induced by Aβ1-42 is also one of the pathological changes of AD. Paeoniflorin can pretreatment inhibit Aβ1-42 induced microglia (TNF)-α, interleukin(1L)-1β and 1L-6 production; at the same time inhibit the nuclear translocation of NF-κB p65 subunit and IkBa phosphorylation ; Reduce the release of CXCL1 and CCL-2; inhibit the increase of vascular endothelial growth factor and its receptor 1 (Flt-1).

Paeoniflorin can also reduce the ischemia-reperfusion injury induced by cerebral infarction in rats. Its pretreatment and post-modeling treatment can reduce the ratio of cerebral infarct areas. Preprocessing can also reduce neurological deficit points. Microvascular ED-1, 1L-1β, TNF-α, ICAM-1 counts, and the increase of MPO immunopositive cells and apoptotic cells can all be reduced by pretreatment. It can also inhibit the excessive activation of astrocytes, nerve apoptosis, up-regulate NeuN, MAP-2 and Bcl-2, and down-regulate Bax, play an anti-apoptotic effect, thereby reducing cerebral ischemic damage. Paeoniflorin also protects rat brain damage caused by ischemia by inhibiting MAPKs/NF-κB-mediated inflammatory damage, and can significantly inhibit the excessive activation of astrocytes and microglia caused by transient middle artery ligation (MCAO) , And prevent the up-regulation of inflammatory cytokines (TNF-α, 1L-1β, iNOS, COX-2 and 5-LOX). Long-term use of PEF can inhibit the activation of JNK and p38MAPK, and increase the activation of ERK, and at the same time can reverse the activation of the NF-κB signaling pathway caused by ischemia. In the hydrogen peroxide-treated PC12 cell model, paeoniflorin can inhibit the NF-κB pathway, the expression of 1L-1β and TNF-α by scavenging oxygen free radicals and reducing the release of lactate dehydrogenase, thereby preventing apoptosis. Anti-oxidant damage.

In summary, as one of the main components of Chinese peony, paeoniflorin (PEF) has a variety of biological activities such as improving blood circulation and immune regulation. It has shown anti-inflammatory and antioxidant effects in a variety of animal models. Inflammation is an important part of secondary injury. A large number of studies have shown that PEF has a powerful neuroprotective effect and plays an important regulatory role in its pathogenesis. The molecular mechanisms involved in the research are often closely related to related inflammatory signaling pathways. Studies have shown that the neuroprotective effect of PEF may be related to the anti-inflammatory and anti-apoptotic effects mediated by TLR4/NF-κB. However, the potential mechanism of PEF on spinal cord injury (SCI) needs further study. Therefore, this article is to establish a rat spinal cord injury model to observe the effects of AT/mTOR and MAPK/ERK signal pathways activated by PEF on nerve regeneration and functional recovery in rats with spinal cord injury.

2. Experimental process

2.1 Animals and groups

75 male SPF Wistar rats, weighing 250-280 g, were purchased from Kunming Institute of Zoology, Chinese Academy of Sciences. All animals were placed in a constant temperature (22±1°C), constant humidity (55%~65%), ventilated, dry, and quiet environment, alternating day and night for 12 hours, eating and drinking freely, and cleaning the cage every 2 days. The experimental rats were tested after two weeks of acclimatization. All experimental rats were randomly divided into 5 groups of OUPS groups (n=15): sham operation group, spinal cord injury model group, SCI+30mg/kg PEF (low dose), SCI+60mg/kg PEF (medium dose), SCI +90mg/kg (high dose). Before the experiment started, the study was approved by the Ethics Committee of Yunnan Open University.

2.2 Establishment of rat model of spinal cord injury

All rats were anesthetized with sodium pentobarbital. In a sterile environment centered on the T10 spinous process, cut about 2cm along the longitudinal line of the spinal cord, excise the muscles on the T10 vertebral body, remove the lamina, and fully expose the spinal cord as the impact area. Except for the sham operation group, the rats in the other groups hit the spinal cord with a weight of 30 g, and then stopped bleeding. After operation, 1×105μL penicillin sodium was injected intraperitoneally to prevent postoperative infection and analgesia. Penicillin 8×104U was intraperitoneally injected 3 days after operation to prevent infection. Manual urination was continued for 8 hours after the operation until the urination function of the rats recovered automatically. On the 3rd day after operation, PEF was injected intraperitoneally at a low dose of 30 mg/kg, a medium dose of 60 mg/kg, and a high dose of 90 mg/kg.

2.3 Basso Betty Bresnahan (BBB) scoring and opening test

Use the blood-brain barrier to check the motor function of the hind limbs. The tests were carried out at 0d, 1d, 7d, 14d, 21d and 28d. Hind limb motor function is divided into 0-21 points according to the 22-level BBB score. A score of 0 means

that the hind limbs are incapable of movement, and a score of 21 means that the hind limbs are completely normal.

Field experiments are a method to evaluate the autonomous activities, exploratory behaviors and nervousness of experimental animals in a new environment. The number of grid crossings and the number of uprights can reflect the degree of autonomous activities and limb activities of rats. In the experiment, the rats were placed in a central grid in an open field. After allowing them to acclimate for 2 minutes, the number of grid crossings and the number of erections ED in the following 4 minutes were recorded, and the degree of mobility impairment of the experimental rats was evaluated.

BBB score and open field test results show that PEF can significantly improve the neurological function of SCI rats. BDNF and NT-3 increased in sham group, BDNF and NT-3 increased in SCI group, and BDNF and NT-3 increased in SPF group. The levels of TNF-α, IL-1β and il-6 in the SCI group were significantly higher than the false ones, while il-10 was significantly lower, indicating the inflammatory state of SCI rats. After continuous administration of PEF, the relative mRNA expression of ERK and p38 was significantly down-regulated, indicating that PEF can significantly regulate the MAPK/ERK signaling pathway. Compared with the sham group, the expression levels of p-AKT/AKT, p-mTOR/mTOR protein and AKT and mTOR mRNA in the SCI group were significantly reduced. When PEF (60 mg/kg and 90 mg/kg) was administered, the levels of these proteins and mRNA increased significantly.

3. Conclusion

From this study, PEF can play a role in SCI through MAPK/ERK and Akt/mTOR signaling pathways.

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